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THE CHOICE OF DRINKING WATER BY THE HONEYBEE

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INTRODUCTION

During the spring and early summer months, bees collect large quantities of water for use in the hive for such purposes as softening down winter stores, etc.

Hertz (1935) showed without doubt, by means of standard training table experiments, that the honeybee can find pure water by "scent" alone, i.e. that they can distinguish the presence or relative proximity of water by means of the relative water-vapour content of the air. Her experiments give only an approximate indication of how far the "scent" can be distinguished, since the distance from the water surface to the bees is only one of the factors responsible for the water-vapour content of the atmosphere at that distance. Although she demonstrated that bees are sensitive to the water-vapour content of the air, it should not, of course, be assumed that water is sensed by the bee as a "smell". We have no evidence that indicates that the receptors involved are the same as those involved in the perception of ethereal oils, or even that the process is chemical rather than physical. It is not to be doubted, however, that it is a true perception by means of sense organs situated in some portion of the body of the bee. The experiments of Hertz were not concerned as are the ones about to be described, with the water *requirement* of bees.

It is well known that honeybees tend to collect water from many undesirable sources, such as rain-water gutters that are choked with decaying organic matter, on the puddles that form on the top of cow dung and sewage effluent, rather than from a source of clean water provided in the apiary for their use. It was, therefore, considered to be of interest and importance to discover what it is that attracts bees to such sources of water. Obviously something more than the sense of appreciation of the presence of water, as described by Hertz, is involved. Many observations and a few experiments had already been made on this subject, and the results, although somewhat conflicting, tended to show that bees prefer saline water to pure water. For instance, Herrod-Hempsall (1931) and Harrison (1932) both produced evidence to show that bees will readily collect water that is nearly saturated with sodium chloride; on the other hand, Betts (1932), Preuss (1919) and others, came to the conclusion that 1·5% sodium chloride is the greatest concentration palatable to bees and about 0·1% is the optimum concentration. Most observers, in fact, tend to explain the choice of drinking water by the honeybee in terms of the salt content of that water. There are, however, at least four major factors that are likely to be involved: sight, the water perception sense described by Hertz (1935), a perception of various olfactory substances contained in the water, and a gustatory sense once the water has been reached. External physical factors, such as temperature and degree of illumination, also undoubtedly play their part but have been neglected for the purpose of the present study, as they did not vary very greatly throughout each experiment.

METHODS

In order to make this study a "training table", similar to that used by von Frisch and Hertz in so many of their experiments on the senses of the honeybee, was set up in a sunny part of the apiary early in the spring of 1939. This table was painted all over with a matt black enamel in order to conserve as much as possible the heat of the sun's rays, and thirty-six shallow glass Petri dishes 4 in. in diameter were arranged on it in the form of a square. Each dish contained a small quantity of washed dry sand. The bees were first trained to come to this table by offering them very dilute sugar syrup on the sand in all the dishes. Once the bees became used to visiting the table these dishes were replaced by others in which the sand was kept moistened with distilled water. At the same time as many as possible of the natural sources of drinking water, such as rain-water gutters, in the immediate neighbourhood of the apiary were treated with strong lysol or carbolic in order to repel bees from them, thus compelling most of the bees to visit the table in order to collect the water necessary for the requirements of their colonies. After a few days, when the bees had become accustomed to collecting their drinking water at this source, the experiments proper were commenced. By this time thousands of bees were visiting the table daily if weather conditions were favourable. Six different substances at a time were tested against one another on this table and six dishes of each substance were arranged in the form of a Latin square, thus rendering statistical analysis of the results a simple matter. It was found that this modification of the technique usually used in this type of experiment greatly expedited matters, since the significance of the results obtained in an experiment could be checked immediately and the necessity or otherwise of further repetition determined. Six of the dishes on the table in each experiment contained distilled water, so that all the results obtained could be expressed relative to the honeybees' partiality for this substance.

If the substances to be tested were solid salts they were dissolved in fresh istilled water, and sufficient (10 c.c.) of the substance to be tested was poured into ach dish to moisten the sand thoroughly, without allowing any of the solution to emain standing on the surface. The dishes themselves were replaced with clean nes fairly frequently in order to minimize, as far as possible, the chance of a bee electing a particular dish because of any odour left on it by bees that had previously isited it. The whole table was turned round through 90° at intervals in order hat bees should not become conditioned to the position of a particular dish ontaining some substance they favoured. There is little doubt that the presence f a large number of bees on a dish, perceived by a flying bee both visually and y an olfactory sense, tends to attract others, and to overcome this to some extent he table was frequently cleared of bees throughout the course of an experiment.

The table with the substances to be tested was put out in place of the table ith the dishes of distilled water, and every 10 min. a count was taken of the umber of bees drinking at each dish. It was found that a bee spends on the verage between 3 and 5 min. collecting a load of water, thus by taking counts at 0 min. intervals the same bee was unlikely to be counted twice when on the same ourney. Frequently the counting had to be done photographically owing to the arge number of bees present at one time and the number of bees alighting and aking off from the dishes.

At the end of an experiment the experimental dishes were again replaced with thers containing distilled water, and care was taken to see that there was always bundant distilled water on the table between experiments, since these extended ver a period of some weeks.

EXPERIMENTS, RESULTS AND CONCLUSIONS

Two types of experiments were undertaken. First, experiments to determine f the honeybees were seeking water for the substances it might contain in olution and, if so, what those substances were and what was their optimal conentration. Secondly, to try to determine why the honeybee tends to prefer tainted vater to pure water, i.e. whether it requires the contained substances or is merely ed thither by an olfactory response to these substances.

The following tables clearly show the response of the honeybee to various rganic and inorganic salt solutions and to other more complicated substances. These salts were chosen because it was thought that they were likely to be essential o the development of the honeybee and that the water collected might be the chief ource of them. In addition, they had all been advocated by practical beekeepers or addition to apiary drinking fountains. As will be seen these two types of xperiments overlap to a considerable extent.

In Table I are shown the results obtained in a typical experiment; in this case o determine the preferences, if any, of the honeybee for *N*/10 sodium chloride, V/10 ferric chloride, *N*/10 magnesium sulphate, *N*/10 sodium carbonate and V/10 sodium phosphate to distilled water. All the experiments were conducted n these lines.

Table I. *Latin square showing the results of a typical experiment carried out continuously between 11 a.m. and 3 p.m. on 18 April 1939*

(The numbers below the substances indicate the number of visits paid to them throughout this period)

Distilled water 99	NaCl $N/10$ 80	$FeCl_3$ $N/10$ 20	$MgSO_4$ $N/10$ 3	Na_2CO_3 $N/10$ 0	Na_2HPO_4 $N/10$ 2
Na_2HPO_4 $N/10$ 1	$MgSO_4$ $N/10$ 5	Distilled water 107	Na_2CO_3 $N/10$ 7	NaCl $N/10$ 76	$FeCl_3$ $N/10$ 25
$FeCl_3$ $N/10$ 16	Na_2CO_3 $N/10$ 3	Na_2HPO_4 $N/10$ 0	NaCl $N/10$ 88	$MgSO_4$ $N/10$ 0	Distilled water 100
$MgSO_4$ $N/10$ 1	Distilled water 86	NaCl $N/10$ 91	Na_2HPO_4 $N/10$ 3	$FeCl_3$ $N/10$ 22	Na_2CO_3 $N/10$ 1
Na_2CO_3 $N/10$ 9	Na_2HPO_4 $N/10$ 1	$MgSO_4$ $N/10$ 0	$FeCl_3$ $N/10$ 11	Distilled water 111	NaCl $N/10$ 77
NaCl $N/10$ 85	$FeCl_3$ $N/10$ 27	Na_2CO_3 $N/10$ 4	Distilled water 121	Na_2HPO_4 $N/10$ 0	$MgSO_4$ $N/10$ 4

The results of various experiments to determine the preference of the honeybee for thirteen different salt solutions are shown in Table II.

Table II. *Showing the preference of the honeybee for various salt solutions as compared with distilled water*

(The substances used in each experiment are arranged in order of preference, and the number of visits to each given)

Substances arranged in order of decreasing preference → (The numbers below the substances indicate the number of visits paid to them)						Exp. no.
Rain water from choked gutter 218	Distilled water 128	NH_4Cl $N/10$ 51	KI $N/10$ 27	$CaCl_2$ $N/10$ 13	Na_2CO_3 $N/10$ 6	I
Distilled water 497	$MgCl_2$ $N/40$ 209	NaCl $N/5$ 164	$MgCl_2$ $N/20$ 135	$MgCl_2$ $N/10$ 54	$MgCl_2$ $N/5$ 28	II
Distilled water 624	NaCl $N/10$ 497	$FeCl_3$ $N/10$ 121	Na_2CO_3 $N/10$ 24	$MgSO_4$ $N/10$ 13	Na_2HPO_4 $N/10$ 7	III

From the results shown in Table II it appears that the honeybee prefers distilled vater to any of the salt solutions offered. It was considered, however, that it was mprobable that the optimal concentrations of these salts, from the bee's point of iew, had been used, therefore further experiments were carried out in which the hree salts, sodium chloride, ammonium chloride and magnesium chloride, which t deci-normal concentrations the bees had seemed to prefer to others, were each ffered at five different concentrations. Similar experiments were also undertaken vith potassium iodide, since several beekeepers have claimed that the bees both ike and obtain benefit from this substance when it is added to their drinking vater. The results of these experiments are shown in Table III.

Table III. *Showing the preference of the honeybee for certain concentrations of sodium chloride, magnesium chloride, ammonium chloride and potassium iodide*

Substances in order of decreasing preference → (The numbers below the substances indicate the number of visits paid to them)						Exp. no.
$\mathrm{NaCl}\ N/40$	$\mathrm{NaCl}\ N/80$	$\mathrm{NaCl}\ N/20$	Distilled water	$\mathrm{NaCl}\ N/160$	$\mathrm{NaCl}\ N/10$	I
966	926	759	579	567	387	
Distilled water	$\mathrm{MgCl_2}\ N/160$	$\mathrm{MgCl_2}\ N/80$	$\mathrm{MgCl_2}\ N/40$	$\mathrm{MgCl_2}\ N/20$	$\mathrm{MgCl_2}\ N/10$	II
502	496	234	189	111	57	
$\mathrm{NH_4Cl}\ N/40$	$\mathrm{NH_4Cl}\ N/80$	Distilled water	$\mathrm{NH_4Cl}\ N/160$	$\mathrm{NH_4Cl}\ N/20$	$\mathrm{NH_4Cl}\ N/10$	III
318	290	263	249	210	106	
Distilled water	$\mathrm{KI}\ N/80$	$\mathrm{KI}\ N/160$	$\mathrm{KI}\ N/40$	$\mathrm{KI}\ N/20$	$\mathrm{KI}\ N/10$	IV
130	48	46	28	21	13	

As will be seen from the results given in Table III the honeybee definitely ppears to prefer $N/40$, $N/80$ and $N/20$ sodium chloride, in the order stated, to istilled water, and is probably unable to appreciate the difference between $N/160$ odium chloride and distilled water, but definitely prefers distilled water to $N/10$ odium chloride. Similarly, the honeybee prefers $N/40$ ammonium chloride and robably $N/80$ ammonium chloride to distilled water, and finds it more difficult o distinguish between distilled water and $N/160$ ammonium chloride, or else does ot actively dislike the latter solution.

The honeybee does not appear to distinguish clearly between $N/160$ magnesium hloride and distilled water, but prefers the latter to higher concentrations of this alt. In the case of potassium iodide the honeybee appears quite definitely to dislike ll the concentrations of this substance that were offered to it.

The above results appear to indicate that the honeybee in a state of nature oes not seek water for the contained salts, since none of the solutions tested with he exception of dilute sodium and ammonium chloride solutions had any particular ttraction.

Table IV. *Showing the preference of the honeybee for rain water, cow-dung water and urine and some of their constituents over distilled water*

Substances in order of decreasing preference → (The numbers below the substances indicate the number of visits paid to them)						Exp. no.
Rain-water distillate 678	Rain-water residual salt 343	Distilled water 318	Rain-water distillate + charcoal 316	NaCl $N/10$ 223	NaCl $N/5$ 95	I
Cow-dung water distillate 396	Cow-dung water 371	Cow-dung water distillate + charcoal 243	Distilled water 198	NaCl $N/10$ 132	Cow-dung water residual salts 31	II
Urine distillate 737	Distilled water 408	NaCl $N/10$ 282	NH_4Cl $N/10$ 166	Urine 93	Urine residual salts 21	III

In the next series of experiments, the results of which are shown in Table I above, direct attempts were made to discover what causes the honeybee to tend t prefer rain water from a leaf-choked gutter, water that has collected on top of cow dung and urine to a source of clean water provided in the apiary. That thi preference does exist has been reported by many observers. It has also been note many times in the apiary in which this work was carried out. An instance of bee much preferring rain water from a gutter choked with decaying organic matter t pure water is shown experimentally in Table II, Exp. 1. For the purpose of thes experiments large samples of the three substances mentioned above were collected A sample of each was immediately evaporated to dryness over a very gently heate sand-bath, the distillate being condensed and collected. The minimum of hea was used, since it was regarded as most important to try to prevent the decomposition by heat of any of the contained substances. The residual salts thus produced wer carefully redissolved in a bulk of distilled water equal in volume to the amount o the substance (500 c.c.) originally taken. All solutions were carefully filtered a atmospheric or slightly reduced pressure. These two solutions, the residual sal solution and the distillate, produced from these three substances were then offere to the bees together with distilled water. In every case the bees showed a marke preference for the distillate over distilled water. Only in the case of the rain water where the salt concentration was presumably very low, was the residual salt solution preferred to distilled water.

In the experiments with the urine and the cow-dung water, the original substances were also offered alongside their derivatives. The bees showed a marke preference for the cow-dung water over the distilled water, but, in the case of the urine the bees flew over it in a large group but very few of them collected it. This is in complete accordance with observations made in the field. Although bees have been observed to be strongly attracted by urine they will seldom collect it, unless it is considerably diluted. A few experiments were commenced using different dilutions of urine, but the relatively short period of the season during which large numbers of bees collect water prevented their completion. So far as they went, however, they definitely tended to show experimentally that bees will collect urine that has been considerably diluted in preference to distilled water. The same thing seems to be true of cow-dung water, although the experiment given in Table IV does not show it. Field observations, however, showed that although bees are often strongly attracted to small puddles of water that collect on the top of cow dung they would only collect it from the older pats, by which time it had, presumably, become more dilute. The cow-dung water used in these experiments was collected from old pats that had been exposed to the weather for about 10 days or more.

An interesting feature of these experiments was the result obtained when samples of rain-water distillate and also cow-dung water distillate were allowed to stand with animal charcoal for 36 hr. at room temperature. When the resulting supernatant fluid was offered to the bees it appeared in the case of the rain water that the bees were no longer able to distinguish between it and distilled water, and in the case of cow-dung water distillate the preference was very markedly reduced. It would therefore appear that some olfactory substance or substances contained in the distillates produced from rain water, cow-dung water and urine, is the factor responsible for attracting the bees to these substances in preference to distilled water. Further, in the case of the rain-water sample and to a large extent the cow-dung water sample, these volatile substances can be absorbed on animal charcoal, in which case the resulting supernatant fluid is found to have lost its strong attraction for the honeybee. The conclusion can therefore be drawn that the honeybee is, in nature, attracted to these sources of water supply by the combined influence of the water perception sense described by Hertz (1935) together with a true olfactory sense. Sight may in nature also play a part in water discrimination, though the possible operation of this sense was, so far as possible, eliminated in the experiments described above. Once the honeybee has found a source of drinking water that it prefers to others in the immediate vicinity of its colony, by means of these senses, it is probably kept there by a gustatory sense, since it has been shown that the honeybee prefers sundry dilute salt solutions to pure water. It is almost certain that in this way a complicated conditioned reflex is set up, an expression of the so-called "memory" of the honeybee, which causes it to visit one particular source of water many times in preference to all others. That this conditioned reflex can persist over a considerable period of time is clearly demonstrated by the fact that honeybees will in the spring visit the site of a drinking fountain, that they used in the previous autumn, even though the fountain had been removed.

Table V, shown above, gives a final summary of the order of preferences of the honeybee for various solutions. If an arbitrary factor 10 is used to express the degree of appreciation of distilled water shown by the honeybee, the degree of preference shown for all other solutions used in these experiments can also be expressed by means of a factor. This factor has been calculated by taking the mean number of visits paid in all experiments to each substance and dividing this by the mean

Table V. *Showing the order of preference of various drinking waters. The various drinking waters have also been given a factor which shows the degree of preference compared with distilled water which has been given the arbitrary factor 10*

Substance	Factor
Rain-water distillate	21
Cow-dung water distillate	20
Cow-dung water	19
Urine distillate	18
Rain water from gutter	17
NaCl *N*/40	
NaCl *N*/80	16
NaCl *N*/20	13
NH_4Cl *N*/40	12
Cow-dung water distillate + charcoal	
Rain-water residual salts	11
NH_4Cl *N*/80	
Rain-water distillate + charcoal	10
$MgCl_2$ *N*/160	
NaCl *N*/160	
Distilled water	
NH_4Cl *N*/160	9
NH_4Cl *N*/20	8
NaCl *N*/10	7
$MgCl_2$ *N*/80	5
NH_4Cl *N*/10	4
$MgCl_2$ *N*/40	
KI *N*/80	
KI *N*/160	
NaCl *N*/5	3
NH_4Cl *N*/5	2
$MgCl_2$ *N*/20	
$FeCl_2$ *N*/10	
Urine	
Cow-dung residual salts	
KI *N*/40	
KI *N*/20	
KI *N*/10	1
$MgCl_2$ *N*/10	
Urine residual salts	
$CaCl_2$ *N*/10	0
Na_2CO_3 *N*/10	
$MgCl_2$ *N*/5	
$MgSO_4$ *N*/10	
Na_2HPO_4 *N*/10	

number of visits paid in the same experiments to distilled water—the figure for sodium chloride *N*/40 calculated in this way comes to approximately 1·7. Both sides of the equation were then multiplied by 10 which gives distilled water a factor of 10 in each case, and in the above example gives sodium chloride *N*/40 a factor of 17. In this way, for example, the preference shown by the honeybee for *N*/40 sodium chloride solution to distilled water can be expressed reasonably accurately by the following ratio:

$$\frac{N/40\ \text{NaCl}}{\text{Distilled water}} : \frac{17}{10}.$$

Several of the experiments have been statistically analysed and differences etween substances found to be very significant. Though the remaining experiments ave not been fully analysed, inspection of the figures shows most of the differences o be very clear cut, and there is little doubt of their significance.

SUMMARY

A brief review of the literature on this subject is given. By means of "training able" experiments in which use was made of the Latin square system in order to llow of rapid statistical analysis of the results obtained, it was shown that:

(1) The honeybee prefers dilute sodium chloride and ammonium chloride olutions to distilled water.

(2) It does not prefer concentrations of these salts higher than $N/20$ solutions nd solutions of various other salts to distilled water.

(3) The honeybee appears unable to distinguish between $N/160$ sodium chloride r $N/160$ ammonium chloride and distilled water.

(4) The honeybee is probably largely attracted to such sources of drinking water as rain water from gutters choked with decaying organic matter, sewage ffluent, etc.: by a water perception sense coupled with an olfactory appreciation f various volatile substances contained in these sources of water.

(5) The volatile substances present in the distillates from the various naturally ccurring solutions examined could be absorbed on to animal charcoal to a large xtent, in which case the resulting supernatant fluid was found to have lost its great attraction for the honeybee and was no longer clearly distinguished from distilled water.

(6) The salts contained in these sources of drinking water do not appear to play an important part in attracting the bee thither.

(7) It was found possible to express the preference shown by the honeybee for various solutions by means of numerical factors based on distilled water having an rbitrary factor of 10. Forty different solutions have been arranged in order of preference by this means.

I am greatly indebted to the Statistical Department, Rothamsted Experimental Station, for assistance in analysing the results obtained in these experiments. I should also like to take this opportunity of thanking my predecessor, Mr D. M. T. Morland, who suggested that I should undertake an investigation of this problem, and my colleagues in the Rothamsted Entomological and Bee Research Laboratories.

REFERENCES

Betts, A. D. (1932). *Bee World*, **13**, 35.
Harrison, C. (1932). *Bee World*, **13**, 44.
Herrod-Hempsall, W. (1931). *Brit. Bee J.* **59**, 248.
Hertz, M. (1935). *Z. vergl. Physiol.* **21**, 463.
Preuss, E. (1919). *Arch. Bienenk.* **1**, 18.

A CHANGE IN THE CHROMATOPHORE PATTERN OF CRUSTACEA AT SEXUAL MATURITY

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(With One Text-figure)

I. INTRODUCTION

While studying the chromatophores of the prawn, *Leander serratus*, it was noticed that the ovigerous females possessed certain groups of white chromatophores which were absent in the males or immature females of this species. These chromatophores appeared on the egg-bearing segments after the "breeding-moult", when the pleopods of the females became structurally modified. It has not been previously suggested that the chromatophore pattern of Crustacea becomes modified at sexual maturity, and an investigation was therefore undertaken to determine the occurrence and behaviour of these chromatophores. *Leander serratus*, *L. squilla*, *L. adspersus* and *L. xiphias* were examined at Naples; *Palaemonetes varians* from Plymouth was also examined. In all these species the additional white chromatophores were found in ovigerous females.

II. STRUCTURE AND DISTRIBUTION OF THE WHITE CHROMATOPHORES IN THE PALAEMONIDAE

(*a*) *Immature males and females.* The structure and behaviour of the normal white chromatophores of shrimps and prawns has already been described (Knowles, 1939). Two main types were observed: The first type is polychromatic and contains red and yellow pigments in addition to the white pigment. This type responds as an independent effector to a change of illumination and is not affected by an injection of eye-stalk extract. It is found distributed at regular intervals along the posterior edges of the body segments of *Leander serratus* and *L. squilla*, and assists in the production of the bars of colour found in these species. The second type responds to an injection of eye-stalk extract by contracting. It contains only a white pigment and is found irregularly scattered in the epidermis of *L. adspersus*, *L. xiphias* and *Palaemonetes varians*, influencing the shade of the body colour, but not assisting in the formation of a body pattern.

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(*b*) *Ovigerous females.* The additional white chromatophores which are found in ovigerous shrimps and prawns appear gradually during the early spring as the female approaches the breeding condition. They are situated on the sides of the pleura, at the basal joints of the first four pleopods, and on the expanded external posterior margins of the basipodites of the first three pleopods. The full development of these chromatophores occurs after the "breeding-moult" when the female acquires a number of structural modifications of the pleopods. Thus the precoxae of the first three pairs of pleopods become elongated, the external posterior margins of the basipodites become expanded posteriorly, and a number of new setae tracts, for the protection of the eggs, appear on the first four pleopods (Sollaud, 1923).

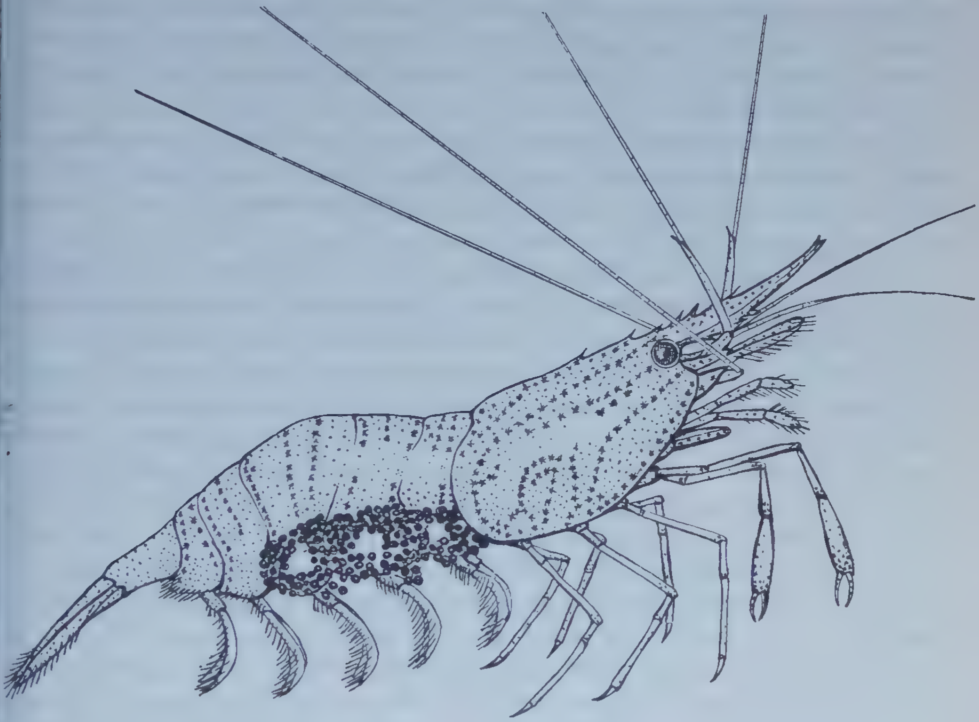

Fig. 1. An ovigerous *Leander serratus*, showing the position of the patches of white chromatophores which appear on the egg-bearing segments during the breeding season. These chromatophores restore the protective pattern which is upset by the presence of eggs.

Only a white pigment can be seen in these expanded white chromatophores of *Leander serratus*, *L. squilla*, *L. adspersus* and *Palaemonetes varians* although traces of a red pigment can be seen when they are contracted. The white chromatophores of *Leander xiphias*, however, all possess a central patch of red pigment.

III. FACTORS INFLUENCING THE DEVELOPMENT AND CONDITION OF THE ADDITIONAL WHITE CHROMATOPHORES OF OVIGEROUS FEMALES

(*a*) *Normal conditions*. The secondarily developed white chromatophores of all the species studied are more sensitive to changes of illumination than to changes of background. They contract sharply in darkness and expand in light, but behave in a more erratic manner on illuminated black or white backgrounds. On an illuminated white background they are always maximally expanded, whereas on an illuminated black background the majority of the white chromatophores on the egg-bearing segments contract. This contraction, however, does not prevent the appearance of the white patches over the eggs formed by these chromatophores (Fig. 1), but tends to minimize their effect.

In their normal behaviour therefore, the secondary white chromatophores of ovigerous females resemble more closely the normal white chromatophores found in *Leander adspersus*, *L. xiphias* and *Palaemonetes varians* than those of *Leander serratus* and *L. squilla*.

(*b*) *Injection experiments*. The white chromatophores situated over the eggs of ovigerous females responded to the injection of an eye-stalk extract in all the species studied. In this respect they differ from the normal white chromatophores of *Leander serratus* and *L. squilla*, but behave in a comparable fashion to the normal white chromatophores of *L. adspersus*, *L. xiphias* and *Palaemonetes varians*.

One-twentieth of a millilitre of an aqueous extract of the eye-stalk of *Leander serratus* was injected into individuals of *L. serratus* and *L. squilla*, from which both eye-stalks had previously been removed. Each injection, which corresponded to one-twentieth of an eye-stalk, was followed by a marked contraction of the white chromatophores situated over the eggs, although the main white chromatophores of the body were not affected by the injection. Therefore, the secondarily developed white chromatophores seen in ovigerous females of *L. serratus* and *L. squilla* are distinct from the normal white chromatophores of these species both in their structure and in their physiological behaviour. However, they resemble the normal white chromatophores of *L. xiphias* and *L. adspersus* and *Palaemonetes varians*, although they are more sensitive to the injection of the eye-stalk extract than are the normal white chromatophores of these species.

(*c*) *Castration experiments*. Females of *Leander xiphias* and *L. squilla* were examined after their ovaries had been prevented from maturing by X-rays or parasites. The largest available individuals were chosen as experimental animals, i.e. in the case of *L. xiphias*, those which had an overall length from the tip of the rostrum to the extremity of the uropods of approximately 6 cm. In *L. squilla* the largest animals obtainable had an overall length of approximately four and a half centimetres.

The ovarian oocytes of a number of these animals were destroyed by X-ray irradiation, some 1–3 months before the breeding season. It was found that one dose of 6800 r. units was sufficient to inhibit vitellogenesis. Post-mortem examination by sections of the gonads of females which had been treated in this way showed that the oocytes had almost without exception been destroyed, and that the gonads consisted simply of a connective tissue stroma. The mortality of experimental animals was high, but about thirty significant results were obtained for each species. These irradiated animals moulted normally, and survived during the breeding season. The irradiated animals and controls were kept living until the first week in May, by which time the controls had all developed the white chromatophores, passed the "breeding-moult", and laid their eggs. In the irradiated animals, however, no ova were laid, *and the white chromatophores did not appear*.

Females, the maturation of whose gonads had been inhibited by parasites, were also examined. *Leander xiphias* is parasitized by *Bopyrus xiphias* (Giard & Bonnier) and *Leander squilla* by *Bopyrus Hellier* (Giard & Bonnier). These Bopyrid parasites interfere with the general metabolism of their hosts by withdrawing blood from the circulation. Very large parasitized females are rare. However, twenty individuals of each species of, or exceeding the sizes given above for the average egg-laying females, were examined. These females did not develop the white chromatophores which are found in the normal ovigerous females; a postmortem examination of their gonads showed that the ovaries of parasitized animals are similar to those found in immature females, consisting entirely of small oocytes without any deposition of yolk.

It was noticed that in females castrated, whether by X-rays or parasites, the suppression of the development of the white chromatophores was not due to a prevention of moulting. The parasitized animals moult much more frequently than normal individuals, while in the case of X-rayed females only individuals which had moulted were considered.

IV. DISCUSSION

It is clear that the white chromatophores which appear on the egg-bearing segments of the female prawn's body are developed in immediate relation to the presence of maturing ovaries, since the suppression of the normal development of the ovaries will prevent the appearance of these chromatophores. This dependence of the appearance of a secondary sexual character on the presence of a normal gonad is a striking and unusual phenomenon for a Crustacean. The change cannot, however, be taken as demonstrating the existence of a "female sex hormone" in these animals, since it is very probable that it is related simply to the metabolic conditions which obtain during yolk deposition. It is not surprising to find that nuptial colorations due to pigment modifications should appear during the internal modifications which accompany sexual maturity. It is probable that the white pigment contained in the chromatophores of Crustacea is guanin, a purine derivative which is found widely distributed in the integument of vertebrates and invertebrates. This substance is known to augment at maturity, and it plays an important part in the nuptial colorations of many fishes, batrachians and reptiles (Verne, 1930). The male of *Triton alpestris* develops a nuptial pattern at maturity due to an accumulation of guanin on the back. *Lacerta viridis* and the teleostean fishes *Gasterosteus aculeatus*, *Perca fluviatilis*, and *Phoxinus laevis* all develop at sexual maturity nuptial colorations of which guanin is an important constituent. It is probable that elaboration of guanin in these forms is due to the violent nucleo-protein movements which occur at gametogenesis.

It is not clear, however, why certain regions of the body in prawns and shrimps are particularly sensitive to the disturbances in metabolism which lead to guanin accumulation, as indicated by the constancy in position of the white chromatophores. The white chromatophores nearest to the eggs are the most affected, and only a small increase is observed in the number of white chromatophores in other parts of the body. Probably sexual maturity leads to a general disturbance of the body metabolism in female shrimps and prawns, but the chromatophores on the egg-bearing segments have developed a greater sensitivity to guanin augmentation because of the special protective coloration which they give. Such groups of white chromatophores situated directly over the gonads serve to mask the eggs and to render males and females less easily distinguishable, besides breaking the continuous line of shadow under the body due to the presence of the eggs (Fig. 1). Since the egg-bearing females are less agile than the males, the development of these white chromatophores at maturity may thus help to protect the females from possible predators.

It is difficult to determine the origin of the white chromatophores on the egg-bearing segments. Possibly they may replace red chromatophores by a substitution or transformation of the red pigment. This suggestion is supported by the behaviour of these chromatophores, which in *Leander serratus* and *L. squilla* function in a manner different from the normal white chromatophores of these species.

SUMMARY

1. White chromatophores appear on the egg-bearing segments of female prawns and shrimps at sexual maturity.

2. Such chromatophores do not appear in the females whose gonads have been destroyed by X-rays or by parasites.

3. It is probable that this appearance of white chromatophores at sexual maturity is correlated with the metabolic changes occurring at yolk deposition in the ovaries.

We should like to take this opportunity to thank Prof. R. Dohrn for the facilities granted us at the Stazione Zoologica at Naples and to thank J. Z. Young, M.A., for his helpful criticism of this paper.

REFERENCES

KNOWLES, F. G. W. (1939). *Pubbl. Staz. zool. Napoli*, **17**, Fasc. 2.
SOLLAUD, E. (1923). *Bull. Biol.* Suppl.
VERNE (1930). *Couleurs et pigments des êtres vivantes*, p. 169. Paris.

THE DAILY RHYTHM OF ACTIVITY OF THE COCKROACH, *BLATTA ORIENTALIS* L.

AKTOGRAPH EXPERIMENTS, ESPECIALLY IN RELATION TO LIGHT

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(*Received 2 January* 1940)

(With Five Text-figures)

I. INTRODUCTION

N the last few years there has been an increasing interest in animal rhythms. arious aspects of the subject have been reviewed by Szymanski (1920), Fox (1923), offmann (1926), Richter (1927), Amirthalingam (1928), Stein-Beling (1935), cott (1936), Gunn *et al.* (1937), Piéron (1937) and Kalmus (1938*a*, *b*). This paper concerned with the daily rhythm in the locomotory activity of the cockroach, *latta orientalis* L. Those who are familiar with this insect in its natural haunts e agreed that it comes out and is active only at night. Can this rhythm of nocturnal tivity be reproduced under experimental conditions and can it be controlled perimentally? Szymanski (1914) and Wille (1920) showed that it could be produced but they did not attempt to control it.

II. APPARATUS AND METHODS

Szymanski (1914) designed several kinds of apparatus, which he called *akto-raphs*, for recording animal activity automatically on a smoked drum. I have sed two practically identical aktographs, one of which has been described in detail unn & Kennedy, 1936). The animal chamber is 20 cm. long and 10 cm. wide; hen the animal walks *along* the length of the chamber, the chamber tips and an tached lever makes a nearly vertical mark on a smoked drum (Gunn, 1937, Fig. 5). ovements of the animal *across* the chamber are not recorded, so that one complete ertical mark on the record represents a walk of at least 20 cm. but probably not sually as much as 30 cm. A spiral drum was used, rotating once a day, so that bout 4 days' records came on to one drum paper. A time marker showed hours.

In the principal experiments, only recently moulted adult cockroaches were sed. Nymphs were kept until they moulted, so that prolonged experiments could e carried out with individual animals before they died of old age. Only males ere used. With certain specified exceptions the work was done in a constant-temperature room at 25·5° C., the normal variation being a short cycle of about ±0·5° C. This is a suitable temperature for behaviour experiments on this species (Gunn, 1934).

In all the experiments, beneath the perforated zinc platform on which the animal walked there was moist common salt, which has an equilibrium relative humidity of 75%. About 30 g. of fresh carrot was put in for food every 3–5 days. This enabled experiments to be carried on with single individuals for as long as 15–20 weeks but, of course, the humidity was disturbed by the carrot. It would naturally be higher near the carrot than elsewhere, and probably higher before the carrot had dried than later on.

When the carrot was renewed, the animal was removed from the chamber, the excess salt solution and faeces pipetted away and more salt was added. The animal was led gently during this exchange and not agitated, and after a few weeks it walked into the removal tube and back into the aktograph, when the time came, in most docile fashion. A few individuals made a temporary escape and had to be handled roughly, but that only occurred early in their case histories.

Thus, apart from the isolation from its kind which was suffered by the cockroach during these experiments, the living conditions were good. I believe that an adult life as long as 22 weeks (19 November 1938 to 24 April 1939) at 25° C. has not previously been recorded for *Blatta orientalis* (see Rau, 1924).

All the apparatus was roofed in and curtained off in a corner of the constant temperature room, and lighted by its own 40 W. electric lamp. This lamp gave a light intensity of about 20–30 metre-candles (1 f.c. = 10·76 m.c.) inside the aktograph, with a narrow shadow at one end and another behind the carrot. This light was controlled by a clockwork switch kindly given by Mr Robert McDowell and it could thus be automatically turned on and off regularly at pre-determined times. When this light was off, it was too dark for the dark-adapted human eye to read the scale of the light meter. In the main room, daylight was always excluded and a 200 W. ceiling lamp was on day and night.

Thus the temperature was uniform and the light on the aktograph was kept uniform or varied at will. The humidity varied, but with a rhythm of 3–5 days as the carrot dried up and was replaced, and with no daily rhythm. The external factor which was not controllable was mechanical stimulation-pressure change, noise and vibration in general. The stands of the aktographs rested on 4 cm. of sponge rubber on a slate slab built into the wall, so gross vibrations must have been rare. The results obtained show that vibration was not a factor of overwhelming importance. The chamber had a glass lid sealed on with vaseline, so draughts were completely absent, and any chemical stimulation from outside must have had a 3–5 day period.

The only other factor which might have affected the activity of the cockroach was the presence of the experimenter and of occasional visitors; these potential disturbances occurred only during the solar daytime, and the complete inversion of rhythm obtained experimentally shows that they were not important. The apparatus normally required no more than a few minutes attention each day, with an hour or so each fourth day.

III. TOTAL ACTIVITY PER DAY

In summarizing the results, the records of complete journeys along the aktograph chamber were counted for each hour of each day; parts of journeys were either ignored or reckoned as whole journeys, according to their length. Two animals gave very long experimental records. They were supplied from Sheffield by Dr Kenneth Mellanby as nymphs, kept at a variable temperature about 25° C. in normal daylight and darkness until their last moult, and then used in these experiments. The distance they walked in a day varied from six to 1300 journeys, with an average of about 150, corresponding very roughly to $1\frac{1}{2}$–300 m. per day (average about 40 m. per day). Fig. 1 shows the relation of this daily activity to the age of the animals and the conditions of light.

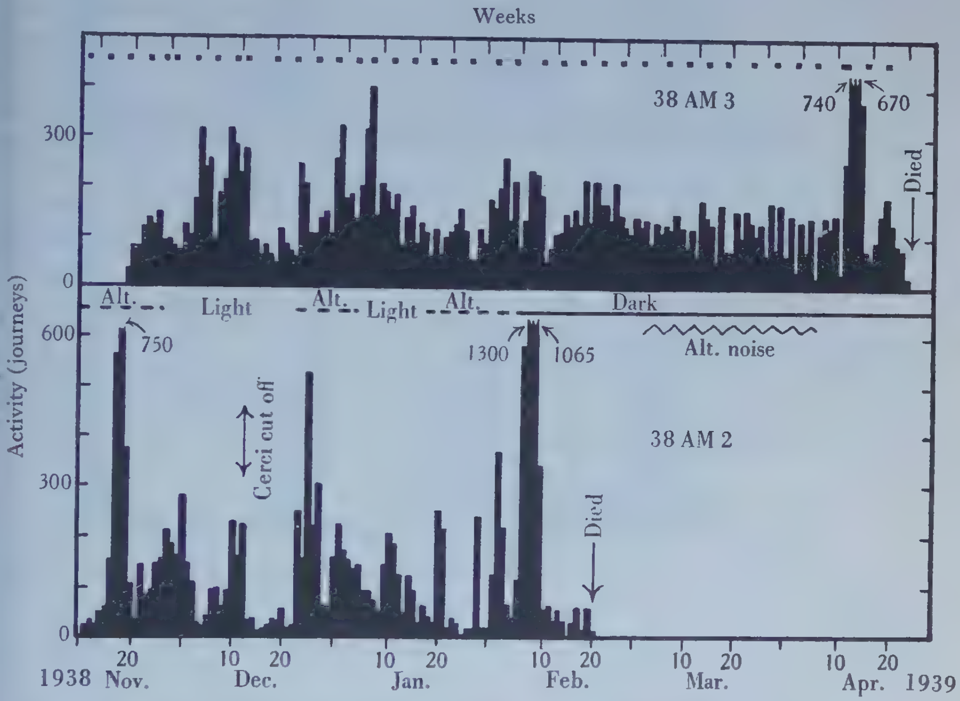


Fig. 1. Daily activity, measured with the aktograph, of two cockroaches from just after the last moult until death. Indications for both parts of the figure: Alt. = alternating light and darkness, 12 hr. each per day; Alt. noise = rattling noise for 12 hr. per day, in continuous darkness; Light and Dark = continuous light and darkness respectively; the spots near the top of the figure indicate the dates on which the food was changed, etc.

It is easy to read several long-period rhythms into these two records, but such rhythms do not coincide for the two animals, and in any case cannot be correlated with any external changes. There are, however, two points of resemblance: for about a week after the cerci of the two cockroaches were cut off (12 December) both animals were rather inactive; in the second place, in each case impending death was signalized by a few days of tremendous activity about a fortnight before death.

It is surprising to find that light had little, if any, effect on the total activity per day. Thus, for these two animals, omitting the periods which were exceptional because of amputations or approaching death, the average number of journeys per day for the whole period was 146 (4876 hr.). The average for alternating light and darkness was 156, for continuous light for periods of upwards of a fortnight at a time it was 158 and for continuous darkness it was 125 journeys per day. The figure for continuous darkness is for one animal only, and that towards the end of its life. For the first month in continuous darkness the average is 145 journeys per day, so it would not be proper to conclude that continuous darkness has any depressing effect.

It can be stated, however, that prolonged illumination under these experimental conditions has no great depressing effect on activity. Neither did the onset of continuous light have any observable effect (Fig. 1). The rise in activity on one of the two occasions when continuous light was replaced by alternating light and darkness can be correlated just as well with recovery from the depressing effect of cercal amputation. Light, then, appears to have no effect on total activity per day but, as is shown below, daily variations in light certainly have a great effect on the time of day at which the activity occurs.

In passing, it may be placed on record that in these experiments one animal walked very approximately 3½ km. (14,500 journeys) in 100 days and the other 5½ km. (22,000 journeys) in 155 days.

IV. DAILY RHYTHM OF ACTIVITY

Each of the two animals dealt with above was first tested in alternating light and darkness, with darkness between 18 and 06 hr. G.M.T. Fig. 2 (*a*, *d*) shows that high activity did not occur at all parts of the day indifferently, but that in this experiment it was concentrated into the hours of darkness. The average figures shown in the graph fairly represent the results for the separate days, except that a few isolated bursts of activity during the period of illumination do not show.

Similar tests were carried out with the dark period lasting for 12 hr. after 11 and 04 hr. G.M.T. daily (Fig. 2, *b*, *c*, *e*, *f*); in these cases the animals had been kept in continuous light for at least a fortnight before the new rhythm of light was started. In all six experiments (Fig. 2) there was one continuous period of high activity each day and the greater part of this period occurred during the hours of darkness.

The average figures shown fairly represent the individual days' records. Thus in Fig. 2*e* high activity is shown as starting between 08 and 09 hr., about 3 hr. before the light was turned out; this actually occurred on 7 out of the 11 days, while on 2 of the other days activity increased 1 and 2 hr. respectively before darkness fell.

In general, high activity started during the first hour of darkness. There were, however, two exceptions to this in the six tests; in one (*e*) it started 2–3 hr. before ark and in the other (*d*) there was no great increase of activity until the third hour n darkness. At the other end, it was usual for activity to reach a fairly low level everal hours before the light came on again. That is to say, activity is brought o an end by some cause other than the change from darkness to light, and it does ot necessarily start when darkness begins. Nevertheless there is a clear correlation

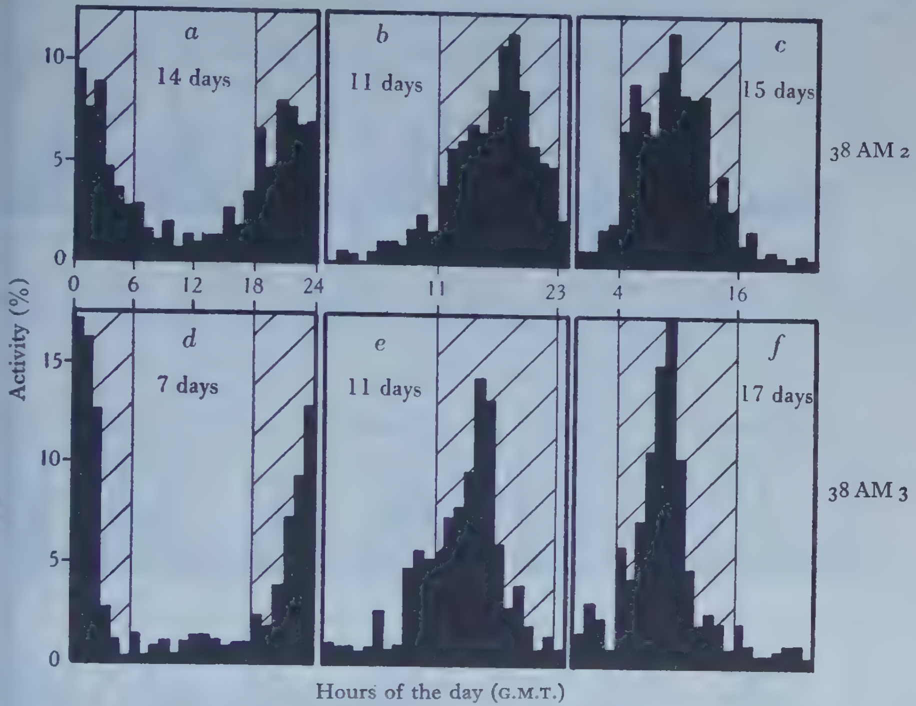


Fig. 2. Hourly activity of two cockroaches in alternating light and darkness (12 hr. each per day). The hours of darkness are indicated by diagonal lines. In order to reduce the six parts of the figure o uniform size, for each experiment the average number of journeys was worked out for each hour of the day, for the 7–17 days, and each average was expressed as a percentage of the sum of the verages.

between the rhythmical occurrence of darkness, whenever that starts during the day (04, 11 or 18 hr.), and the rhythm of high activity. In fact, by suitable adjustment of the time of darkness, the high daily activity of the cockroach can be made o occur when one pleases. Mellanby (1940) has obtained a similar result in a study of cockroaches in a natural infestation. Fig. 3*a* shows that this rhythm starts without delay as soon as continuous light is replaced by alternating light and darkness (see below).

V. ABOLITION OF THE RHYTHM

Since the activity rhythm is related to the rhythm of light and darkness, it might be expected that in continuous light the activity rhythm would disappear. Practically peaking, it does, but not at once (Fig. 3*b*). The rhythm previously established by alternating light and darkness persists for a time but, within the first few days complete hours of the active period pass with no activity at all, while bursts o activity occur increasingly frequently at other times. The process of averaging th results from several experiments conceals these effects (Fig. 3*b*), but they can b seen clearly in the record of a single test (Fig. 4). Continuous darkness (Fig. 4 has much the same effect as continuous light.

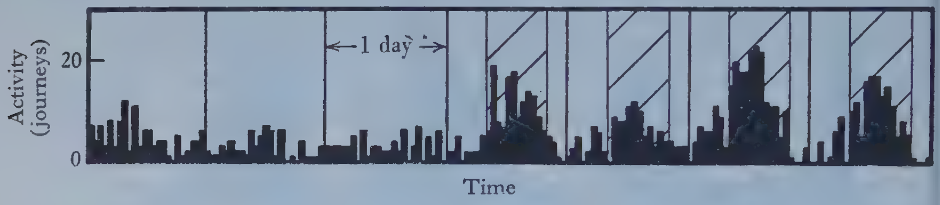


Fig. 3*a*. The initiation of rhythmic activity immediately the alternation of light and darkness begins Average number of journeys in each hour in four experiments which were put together irrespectiv of the time by the clock; the onset of darkness (diagonal shading) on the fourth day was made t coincide for the four experiments.

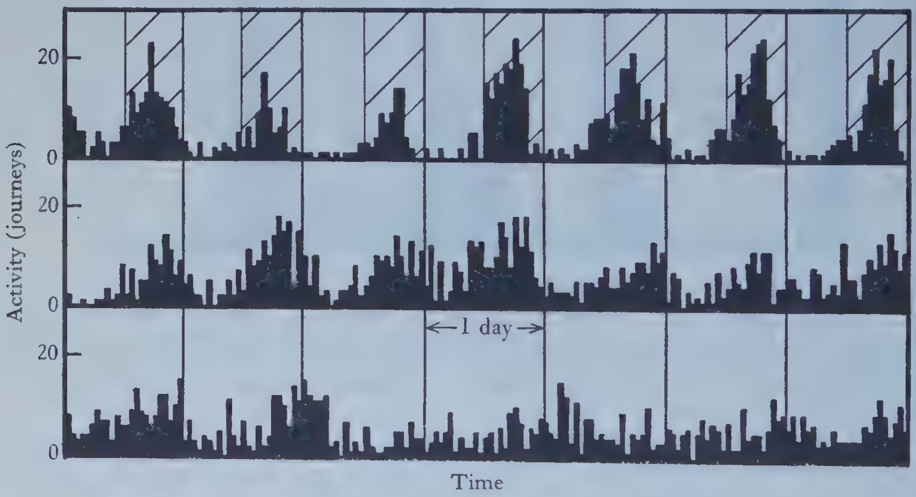
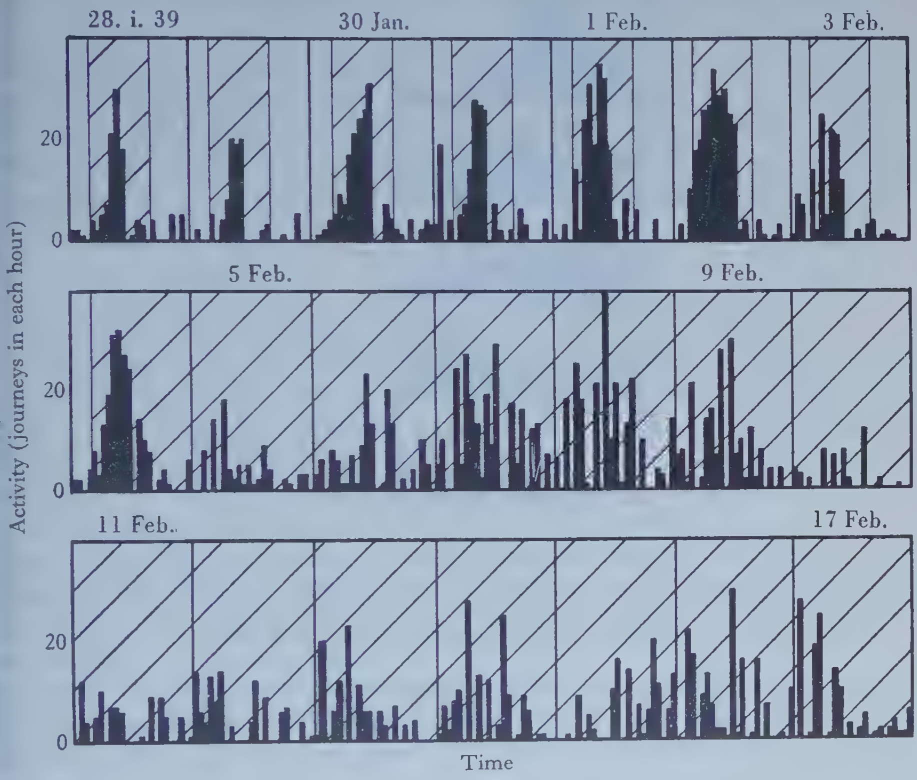


Fig. 3*b*. The slow disappearance of the daily rhythm of activity in continuous light. In the first week shown there was an alternatior of light and darkness. Average of four experiments put together as in Fig. 3*a*. Diagonal lines indicate the dark periods.

After several weeks in continuous light or continuous darkness, the record of a single day's activity no longer gives any indication of the previous rhythm. Even so, the averaging of the daily records of one individual long kept in constant light or darkness reveals some trace of rhythm (Fig. 5). In a selected group of 12 consecutive hours averaged over a fortnight the activity may be twice as great as in the other 12 hr.; but in the proper rhythms, such as are shown in Fig. 2, the difference is of a different order, the journeys in the active period usually numbering at least six times as many as those in the inactive period.

This residual rhythm, if it may be so called, is not detectable by simple nspection of the records, but appears only when the numbers of journeys are veraged for a week or more. It has been found in all the records for continuous ght or darkness. It is characterized by the activity being above average in a series f *consecutive* hours, but only slightly above average. Thus in the four periods in ontinuous darkness shown in Fig. 5, the selected groups of 12 hr. contained 9, 59, 59 and 64% respectively of the day's activity. This active period was



ig. 4. The disappearance of the daily rhythm of activity in constant darkness. Individual record rom animal numbered 38 AM 3. During the first week (top) there was an alternation of light and arkness (12 hr. each) and from 04 hr. on 4 February onwards there was continuous darkness.

etween 03 and 15 hr. in the first two and between 12 and 24 hr. in the last two eriods. During the latter, there was a motor making a continuous rattling noise etween 06 and 18 hr.; but it seems unlikely that this could have affected the nimal, for the aktograph was well buffered and the cerci had been cut off. Hair ensilla on the cerci have an auditory function (Pumphrey, 1940).

The excess over 50% shown by the activity during the 12 hr. period of greatest ctivity is statistically significant. The active period did not tend to fall into the ame part of the day in different experiments, but most of the experiments were ot carried on for long enough to allow the period of slightly higher activity to drift far from the time determined by the previous rhythm of light. It is possib that the repeated process of changing the carrot and cleaning the chamber w responsible for the residual rhythm; this cleaning was done about every fourth da usually between 10 and 12 hr., and the animal was then in the light for under a hour, usually 15–30 min. In any case, such a slight residual rhythm can hardly b regarded as of great importance, and it is clearly distinguishable from the norm rhythm.

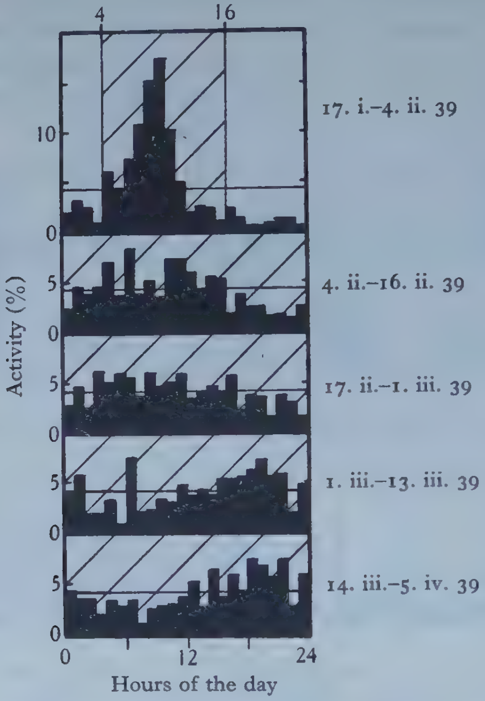


Fig. 5. Residual rhythm of a cockroach in continuous darkness. The top section of the figure show the previous rhythm in alternating light and darkness; the horizontal line on each section show the hourly average. Percentage activity averaged for 12–22 days as in Fig. 2.

VI. OTHER EXPERIMENTS

In addition to the experiments with these two animals, twenty-seven oth animals provided records for a total of 236 days. For a number of the anima these records covered periods too short to give such clear-cut results as have bee described above, but in no case do these records contradict the conclusions alrea drawn. Some of the experiments are, however, worth mentioning.

In an aktograph in a room subject to the ordinary variation of temperature a light, nocturnal activity was observed as usual, but on Sunday night there was n activity. The temperature fell slowly from midday on Saturday to early on Monda morning, reaching a minimum of 12° C. on one of the two occasions; presumab the low temperature prevented activity.

In one experiment lasting 4 days, the 12 hr. darkness was followed each day by only 8 hr. of light, so that darkness came on 4 hr. earlier each day. On the first 2 days activity started 2 hr. after dark, on the third day 4 hr. after dark and on the fourth day 8 hr. after dark. Thus the onset of activity did occur earlier than previously, but it did not shift as much as the onset of darkness.

A number of individuals were first tested in continuous light (25° C.); of these, three trapped specimens which had been kept in an open dish in an ordinary room did not show any rhythm from the start. Perhaps the rise of temperature to 25° C. upset the internal "clock" of these animals. On the other hand, of seven animals which had been bred from the egg inside a dark oven at 25° C., three showed a clear rhythm in continuous light, two a doubtful one, one none at all, while the remaining animal was quite inactive. No doubt the oven was not quite light proof, while all manipulation during rearing—cleaning, feeding, etc.—was carried out in daylight hours, so these experiments cannot safely be used as evidence about the method of acquisition of the tendency to show a rhythm.

VII. DISCUSSION

Szymanski (1914) with *Blatta orientalis* and Wille (1920) with *Blatella* (*Phyllodromia*) *germanica* did ten aktograph experiments each, each with one animal at a time and lasting 24 hr. Both found an average of 3 hours' activity, starting at 15.30 hr. at the earliest and ending at 00.30 hr. at the latest. The temperature varied around 20° C. In my experiments at 25° C. the active period lasted longer than this, being seldom less than 6 hr. Szymanski also recorded a short period of activity at about 06 hr. in the morning, and because of this second period he regarded the cockroach as polyphasic (Szymanski, 1920). Truly polyphasic animals, like mice, have many short periods of activity each day. My experiments indicate that the cockroach is really monophasic.

Neither of these authors attempted to analyse the cockroach's activity rhythm. A number of authors, on largely or entirely theoretical grounds, have considered that diurnal changes of temperature and humidity provide both the stimulus for the rhythm and the evolutionary reason for its existence (Picard, 1912; Necheles, 1927; Crawford, 1934). It may be that factors other than light can be used to control the rhythm, but so far light has been found to be potent and other factors mpotent in a number of cases (Kalmus, 1938*a*, *b*). I hope to investigate these and other questions at a more suitable time. For example, can rhythms of periods other than 24 hr. be set up? Will a short pulse of half an hour's darkness each day suffice to maintain a rhythm? Would rhythms occur if there were no correlation between danger and light in the life of the individual? What is the internal clock, especially for a persistent rhythm? Does the light act through the compound eyes?

Szymanski (1914) carried out two experiments which are of considerable mportance. In one of these, half the aktograph chamber was shaded and half ighted all the time, while in the other, half was warmed and the other half not warmed. In both experiments, during its active period the cockroach walked indifferently in the two halves of the chamber. During this time, the well-known responses to light and temperature seem to have disappeared. Mellanby (1940c found, however, that in a natural infestation—in a less limited space than it available in an aktograph—cockroaches did not emerge into a continuously lighted room. They may have emerged in other rooms nearby or they may have been active inside the cavities in the wall.

Esterley (1917) showed that certain marine plankton copepods tended to come to the top of a long vertical tube in the evening, even though kept in continuous darkness at an even temperature for 2 days. This accentuation of geo-negative behaviour occurred with a diurnal rhythm even in the absence of external clues.

Broadly speaking, it is thus to be expected that many animals which show a diurnal rhythm will behave differently according to the phase in which they are tested. Most of the work on behaviour is done during the daytime, and this work on the cockroach suggests that the nocturnal phase can be investigated without doing more than check experiments at night. In the cockroach, at any rate, the rhythm can be reversed by a suitable alternation of light and darkness and then for a few days the rhythm persists almost in full in continuous light. Experiments on behaviour during the active phase can then be done in the daytime during these few days. In the experiments described here, the institution of a new rhythm was always preceded by a period of continuous light; this may not be necessary.

VIII. SUMMARY

1. In an aktograph at 25·5° C., at upwards of 75% relative humidity and with food present, the average locomotory activity of the cockroach per day does not depend on whether there is continuous light for weeks, or continuous darkness, or a daily alternation of light and darkness.

2. When temperature and humidity do not vary during the day and other factors are kept as constant as possible, the cockroach's activity can be largely concentrated into any desired half of the day, simply by suitably adjusting the time of onset of the half-day's darkness. A rhythm can thus be set up, so that the main activity occurs at the same hours each day.

3. This activity rhythm persists for some days in continuous light or continuous darkness, but eventually activity becomes much more evenly spread over the whole day, leaving only a slight residual rhythm which is unrelated to the previous conspicuous one. A new conspicuous rhythm can then be started at once by alternation of light and darkness.

4. There are indications that animal responses to physical stimuli may depend to a considerable extent on whether the animal is in the active or the inactive phase of its daily cycle. A method is suggested for making it possible to study the nocturnal phase during the daytime.

I have enjoyed the pleasure and benefit of discussing this work with Dr Kenneth Mellanby and of comparing his unpublished results with mine.

REFERENCES

IRTHALINGAM, C. (1928). *J. mar. biol. Ass. U.K.* **15**, 605–41.

AWFORD, S. C. (1934). *Quart. Rev. Biol.* **9**, 201–14.

ERLEY, C. O. (1917). *Univ. Calif. Publ. Zool.* **16**, 393–400.

K, H. M. (1923). *Proc. roy. Soc.* B, **95**, 523–50.

NN, D. L. (1934). *Z. vergl. Physiol.* **20**, 617–25.

— (1937). *J. exp. Biol.* **14**, 178–86.

NN, D. L., JENKIN, P. M. & GUNN, A. L. (1937). *J. Obstet. Gynaec.* **35**, 839–79.

NN, D. L. & KENNEDY, J. S. (1936). *J. exp. Biol.* **13**, 450–9.

FFMANN, R. W. (1926). *Handb. norm. path. Physiol.* **17**, 644–58.

LMUS, H. (1938*a*). *Riv. Biol.* **24**, 191–225.

— (1938*b*). *Z. vergl. Physiol.* **25**, 494–508.

ELLANBY, K. (1940). *J. exp. Biol.* **17**, 278–85.

CHELES, H. (1927). *Chin. J. Physiol.* **1**, 143–56.

CARD, F. (1912). *Bull. sci. Fr. Belg.* **46**, 235–47.

ÉRON, H. (1937). *J. Psychol. norm. pathol.* **34**, 398–412.

MPHREY, R. J. (1940). *Biol. Rev.* **15**, 107–32.

U, P. (1924). *Trans. Acad. Sci. St Louis*, **25**, 57–79.

CHTER, C. P. (1927). *Quart. Rev. Biol.* **2**, 307–43.

OTT, W. N. (1936). *Trans. roy. ent. Soc.* **85**, 303–30.

EIN-BELING, I. VON (1935). *Biol. Rev.* **10**, 18–41.

YMANSKI, J. S. (1914). *Pflüg. Arch. ges. Physiol.* **158**, 343–85.

— (1920). *Z. allg. Physiol.* **18**, 105–62.

ILLE, J. (1920). *Biologie und Bekämpfung der deutschen Schabe* (Phyllodromia germanica *L.*) Pp. 140. Berlin: Paul Parey. Beiheft zu *Z. angew. Ent.* **7**.

THE DAILY RHYTHM OF ACTIVITY OF THE COCKROACH, *BLATTA ORIENTALIS* L.

II. OBSERVATIONS AND EXPERIMENTS ON A NATURAL INFESTATION

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The University of Sheffield

(*Received* 27 *December* 1939)

(With One Text-figure)

INTRODUCTION

For some years I have been studying the reactions of various insects infesting room in an animal house. The two commonest species are the bed-bug, *Cime lectularius* L., about which some information has been published (Mellanby, 1939) and the cockroach, *Blatta orientalis* L., which forms the subject of the present pape The remaining arthropod fauna includes small numbers of silver fish, beetle crickets, flies and woodlice.

The room has been described in detail elsewhere (Mellanby, 1939). It is used fo keeping and breeding experimental rats, and racks of cages cover three walls. Th main food of the cockroaches falls from the rats' cages. There are windows alon the top half of one wall; they admit sufficient light for working, but not direc sunlight, and the intensity of illumination during the day is usually between 30 an 50 metre-candles (1 m.c. equals 0·093 f.c.). The room is also lighted electricall and so different degrees of illumination can be produced. The floor is covered wit concrete and the walls with smooth plaster; during the day the cockroaches liv inside the walls, and can enter and leave by the spaces around the steam heatin pipes. The temperature is usually between 20 and 25° C.

The animal house under consideration contains a number of rooms, but I hav only made an extensive study of one of them. There does not appear to be a grea traffic of cockroaches between the rooms (all are infested), but there may be some. I those experiments in which the room was kept illuminated for periods of severa days, some of the cockroaches normally infesting it may have migrated to other part of the animal house and so been subjected to different conditions. For this reaso certain conclusions expressed here must be accepted with some caution.

Trapping was done using ordinary "Demon" cockroach traps. It was foun that equally large numbers of insects were caught if no bait was used, so the trap were not baited. On a large number of occasions the traps were left in the roo from about 5 p.m. G.M.T. until about 9 the next morning. On other occasions they were examined at 3-hourly intervals, when the traps were removed from the animal room, emptied and returned within 5 min. without switching on any light.

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[1] Sorby Research Fellow of the Royal Society.

During the course of this work I have been in touch with Dr D. L. Gunn, who has been studying the daily activity of the cockroach in the laboratory. He has made a number of valuable suggestions for further experiments in the animal room. Some of his results are given in this *Journal* in the paper immediately preceding this, and in both our papers the laboratory experiments and the observations made on the natural infestation are compared. The fact that there is so much agreement in the final conclusions is gratifying.

NORMAL PERIODICITY

No healthy cockroach was ever seen in the animal house during the day; occasionally a moribund individual was found which had apparently been unable to crawl back to shelter. If the light was switched on at any time between 10 p.m. and midnight, hundreds of the insects were seen all over the room. When the light was put on they all ran rapidly, first to the area partly shaded by the cages and then into the cracks in the walls. The open floor was always clear of cockroaches within half a minute of switching on the light.

The results obtained by making catches over 3 hr. periods on 12 nights spread over 9 months are given in Fig. 1. The total cockroach population varied during the period of the experiment, and the catches made during these nights ranged between 127 and 17 individuals; the average figure was 48 (S.E. mean ± 8). This includes adults and all stages of nymphs, for analysis of the figure shows no significant differences between the behaviour of the different stages. To make the results comparable, the figures for each 3 hr. period are given as a percentage of the night's total catch.

It will be seen from Fig. 1 that the cockroaches were most active during the first half of the night, and that fewer were caught during the second half although it was still equally dark. Thus, of the total number, 52·4% were captured before 23·00 hr. (11 p.m.), and only 5·6% were captured after 2 a.m. Activity appears normally to take place only during darkness. In summer, when the nights were short, activity started soon after sunset, but in winter it had been dark for some hours before full activity was exhibited. Both in summer and winter there was practically no activity during the later hours of darkness.

Changes in temperature are indicated for all occasions in Fig. 1, and fluctuations of humidity are also shown in the majority of cases. There appears to be no direct correlation between cockroach activity and either of these climatic conditions. The temperature was always such as to allow activity to take place (see Gunn, 1934), and whether it rose or fell during the night activity took place at approximately the same time. The humidity varied approximately inversely with the temperature, a rise in temperature having little effect on the absolute humidity and thus causing a fall in the relative humidity (this could also be measured as an increase in the saturation

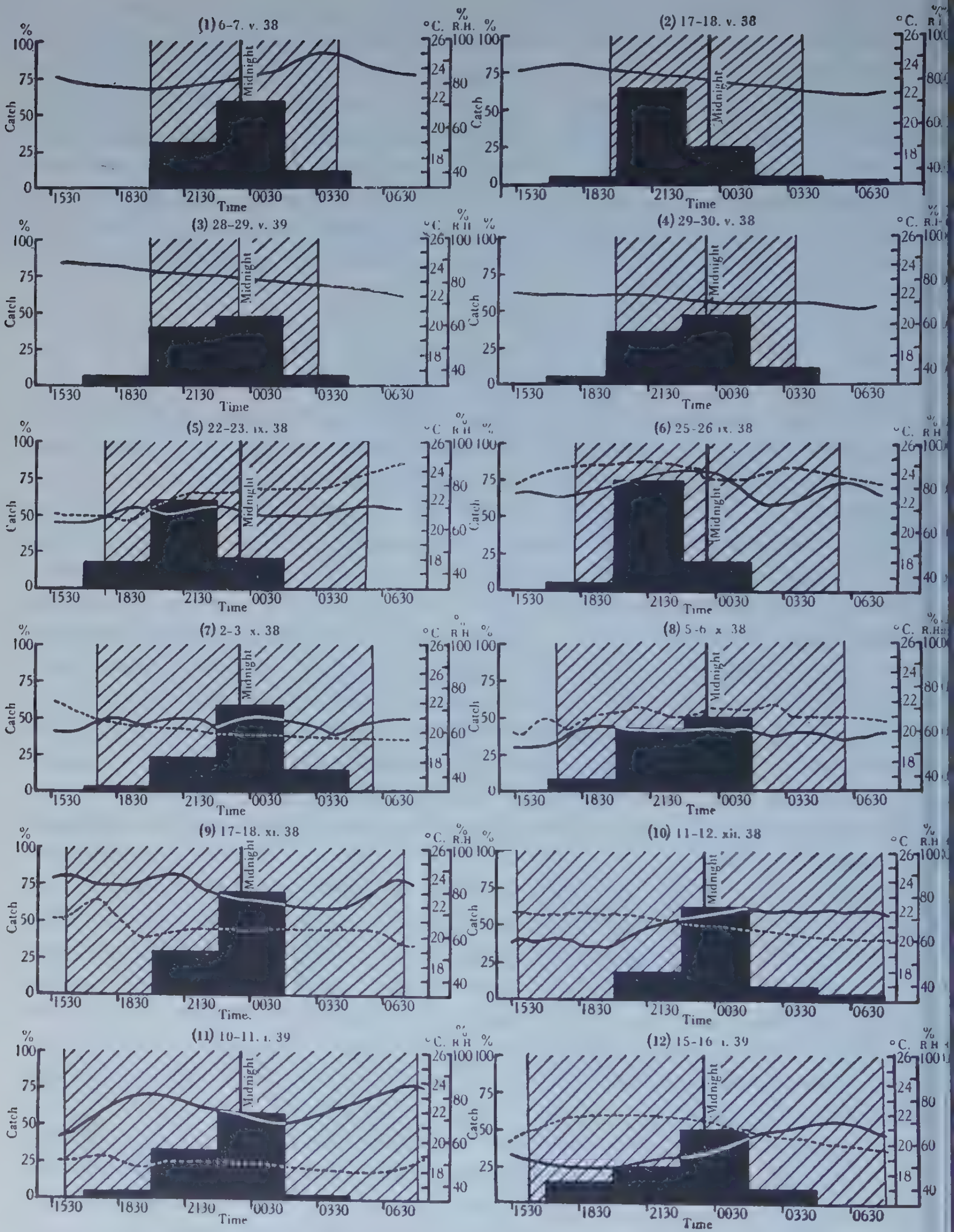
(1) 6-7. v. 38
(2) 17-18. v. 38
(3) 28-29. v. 39
(4) 29-30. v. 38
(5) 22-23. ix. 38
(6) 25-26 ix. 38
(7) 2-3 x. 38
(8) 5-6 x. 38
(9) 17-18. xi. 38
(10) 11-12. xii. 38
(11) 10-11. i. 39
(12) 15-16 i. 39

Fig. 1. Numbers of cockroaches captured in traps examined at 3-hourly intervals, each diagram showing results obtained over one 24 hr. period. Black area represents the number of cockroaches. Period from sunset to sunrise (G.M.T.) shaded diagonally. Continuous line = temperature. Broken line = relative humidity. In each diagram the horizontal scale gives the time, the vertical scale on the left the percentage of the total catch of cockroaches at any period, and the scales on the right the temperature (° C.) and relative humidity (%) respectively.

deficiency). Therefore if the activity of the insects is not correlated with the changes in temperature, it could hardly be expected to be correlated with humidity either. Activity seems to take place at approximately the same time at all seasons of the year, independently of fluctuations of temperature and humidity, and of changes in the length of day.

It is interesting to compare these results with those which Gunn obtained in experiments where conditions of illumination simulated those of night and day (see p. 270). His results bear a striking resemblance to mine—maximum activity during the earlier hours of the "night", and little during the later hours of the night or during the "day".

EFFECTS OF CONSTANT ILLUMINATION

When the room was illuminated by a 60 W. electric bulb, the intensity of light in the open varied between 12 and 6 m.c., but below the cages it was less than half a metre-candle. In a total of 28 nights under these conditions, on 22 no cockroaches were captured, and on the remaining 6 nights a total of only 8 individuals was trapped (the average figure for 42 catches made in darkness in the period covered by this experiment was 65). On numerous occasions when I entered the illuminated room between 10 p.m. and midnight, I never saw a single cockroach. The few that were caught probably ran along to the traps in the shadow under the cages (two of the traps were actually in this shadow). Even after several days of constant light, no activity was observed among the cockroaches. It was thought that perhaps the light might not be so much preventing the insects from leaving their holes as diverting them into other parts of the animal house. To get over this, all the rooms were illuminated for a period of 8 days. Even after this no cockroaches were observed in any of the rooms. It is, of course, not known how active they were inside the walls, or whether they found any food there. If it were possible, it would be interesting to continue this experiment for a longer period (many weeks), until the insects in the walls would be suffering from extreme starvation. Various circumstances make it impossible for me to do this.

Some experiments were made to find whether a very dim light might be left burning without preventing the activity of the cockroaches but still sufficiently bright to allow the insects to be observed. As the cockroach population was fluctuating, it was impossible to express the results of the various illuminations upon the activity other than in a rather rough manner. With a 15 W. lamp, which gave an illumination of 2·5 m.c. in the middle of the room, few cockroaches were caught (about one-tenth the number captured in darkness). A smaller light which gave an intensity of only 0·6 m.c. in the middle of the room, and of only one-twentieth of this under the cage racks, affected the activity of the insects less. On four nights during one week, on two occasions when the room was in darkness the catches numbered 113 and 211, and on two occasions with the small lamp burning the catches were 55 and 39. These figures show that further laboratory experiments under more carefully controlled conditions might yield valuable results, but that it is impossible to leave a lamp burning which gives sufficient light to allow observation of the insects without affecting their activity, for even with an intensity of illumination of 0·6 m.c. it is difficult to study the cockroach's activity.

Gunn (1940) has found that constant light of an intensity higher than that produced by the 60 W. lamp in the animal house still allows the cockroach to exhibit activity in the laboratory. In his experiments the insects were unable to move out of the light, and for a time they then exhibited periodic activity not unlike that observed in alternating light and darkness. In the animal house when it was possible for the animals to choose between the darkness (inside the walls) and the light (in the room), they chose to remain in the dark, though perhaps they were very active inside the walls during the normal period.

EFFECTS OF CONSTANT DARKNESS

It was possible to darken the animal room during the day by means of shutters fitted over the windows. As the rats had to be fed, it was never possible to keep the room permanently darkened for more than 48 hr., but on one occasion during 4 days the light was only put on for about half an hour on each morning.

When the room was darkened earlier than usual, provided that during the previous night normal conditions of darkness had existed and the room had not been artificially lit up, no effects on the cockroaches were observed. They began their activity at the same hour as they would have done had the room only become dark after sunset. This was observed on a number of occasions when the room was darkened at noon, and even when it was kept dark for 48 hr. no effects were observed. It is possible that further darkness might have upset the rhythm, but because of the rats it was impossible to maintain such conditions.

Darkness at an abnormal time following abnormal illumination had a marked effect on cockroach activity. When the room had been illuminated for 8 days, during the whole of which period only 4 cockroaches had been trapped, and was then darkened at 12.50 (p.m.), 40 cockroaches were captured within 2 hr. As mentioned above, darkening the room at this time after a night of darkness did not make the onset of activity any earlier. Then when the room was kept illuminated until 04.30—by this time all activity would normally be over (see Fig. 1)—and then darkened for 4 hr., 42 cockroaches were captured. These experiments seem to indicate that there was little movement of the cockroaches into the other rooms, and that they were being prevented from coming out of the walls by the light. In fact they almost appear to have been "waiting" there for suitable conditions for activity, i.e. darkness.

It has been noted that darkness following abnormal illumination may reveal an upset in the normal rhythm of activity of the cockroach. But as soon as normal conditions of illumination by day and darkness by night return, these insects appear to revert to their original periodicity, and thereafter early darkening of the room has little effect. After a period of several days of constant illumination, 3 days of normal illumination occurred, and then the room was darkened at mid-day. Only 3 cockoaches were caught before 17.00, 11 before 20.00, and 75 were captured during the ight. The darkness was maintained for a further 24 hr., and no cockroaches were apped up to 17.00 hr., 8 were trapped before 20.00, and 76 after this hour. These gures indicate not only that a few days' exposure to normal conditions practically e-establishes the activity rhythm of the cockroach, but also suggest that under onditions of constant darkness the rhythm may establish itself further. That this eally does happen is further suggested by some observations made during the eriod of 4 days' darkness after constant light lasting 8 days. During the first 24 hr. f darkness, the majority of the cockroaches (64 %) was captured before 20.00 8 p.m.), and only 36 % after that hour, though normal activity is confined to this ater period. During the remaining 3 days of the experiment, although irregular ctivity was noticed, more than 60 % of the cockroaches were captured during the eriod in which activity is normally manifested under natural conditions of illumina-on.

EFFECTS OF MECHANICAL DISTURBANCES

These experiments were made to see how vibration affected the activity of the ockroaches. After preliminary trials it was found simplest to produce the vibration y walking noisily about the room instead of spending time devising mechanical pparatus.

As mentioned above (p. 279) if the room was illuminated suddenly at 22.00 hr. undreds of cockroaches were seen running about the floor. If, before switching n the light, I walked about the room for 5 min., then practically all the insects isappeared. However, the cockroaches appeared to "get used to" a disturbance of his kind, for if I walked about the room for an hour instead of 5 min. before switching n the light, then a large number of the insects had reappeared. Trapping experinents confirmed this conclusion for similar numbers were captured between 0.00 and 23.00 hr. whether or not the experimenter walked about the room for the vhole period.

Cockroaches are known to be sensitive to air movements (Necheles, 1924), so ome experiments were made using an electric fan. When this was pointed at the eiling, so that all the air in the room was kept moving but there was no considerable raught along the floor, the catch was not affected. When the fan blew directly at he traps some effect was noticed. On six consecutive nights experiments were nade. On days 1, 3 and 6, when the fan was not running, 116, 87 and 113 (mean = 05) cockroaches were captured: on the other nights, when the fan was blowing irectly at the traps, the catches were 42, 46 and 77 (mean = 55) cockroaches espectively. The air velocity in the neighbourhood of the traps was approximately o m. sec., which is considerably higher than these insects are likely to meet ormally. If so great a wind only reduced the catch by about 50 %—and it seems probable that it did not reduce the activity of the cockroaches at all, for they presumably were more concentrated in other parts of the room—then it is unlikely hat air movements have much effect on the general activity of the insects, though udden changes may have temporary effects on the behaviour.

All these experiments on the effects of mechanical disturbance show that thes factors are certainly of much less importance than light in maintaining or upsettin the activity rhythm of the cockroach.

FURTHER OBSERVATIONS ON THE EFFECTS OF ATMOSPHERIC HUMIDITY

It has been suggested that cockroaches are most active at night, not because i is then dark, but because the humidity is usually higher (Necheles, 1927). Th results given in Fig. 1, showing no correlation between normal activity and atmospheric humidity, contradict this view. On several occasions I sprayed and sprinkle water in the animal room in the evening, and by these means produced a practicall saturated atmosphere. If the room was then kept dark, a normal catch of cockroaches was obtained, but if the light was left burning the activity of the insects wa entirely prevented. Also, on those occasions when the humidity rose considerabl during the day, no activity was then found. It appeared unlikely that atmospheri humidity has any important effect on the activity rhythm of the cockroach.

DISCUSSION

The results given in this paper indicate that in a natural infestation unde normal conditions of illumination, cockroaches are mainly active during a perio of about 6 hr. during the 24, and the peak of this activity occurs shortly befor midnight. Provided that the temperature falls within the zone of normal activity its fluctuations have little effect on the periodicity of the activity. The activity occur only in the dark, but seasonal changes in the length of day and night have no grea effect on the time at which activity starts and stops. The rhythm appears to b controlled in the cockroach by some internal mechanism, as to the nature of whic we at present have no knowledge.

It is interesting to compare my results with those obtained by Gunn in th laboratory. Our techniques were entirely different—my results were obtained b trapping a sample of a wild population under more or less uncontrolled conditions Gunn (1940) worked in each experiment with one insect and he controlled all suc conditions as temperature, humidity, illumination and food. Nevertheless ou results are strikingly similar, except for the fact that he found that activity continue in the light whereas in my experiments this never happened. This point is discusse above (see p. 281). All the other observations on the periodicity of activity made b the two methods show a striking similarity, and suggest that rhythmic activity is factor inherent in the cockroach.

SUMMARY

1. Trapping experiments were made to investigate the activity of cockroache in a naturally infested animal room.

2. Under normal conditions of illumination (light by day and dark by night)

activity only occurred at night, and most activity occurred during the period just before midnight. There was little activity during the later hours of darkness.

3. Artificial illumination of the order of 6 m.c. almost entirely prevented the cockroaches from appearing. Even lower intensities of light greatly reduced the number which appeared.

4. Darkening the room during the day did not cause the cockroaches to appear, unless the room had been artificially illuminated during the previous night.

5. Mechanical vibration and air movements appear to have little effect on the rhythmic activity of the cockroach.

6. The rhythmic activity of the cockroach appears to be controlled by some internal mechanism which we do not yet understand. External factors like light can modify the rhythm and may temporarily abolish it, but under normal conditions it is readily re-established.

REFERENCES

Gunn, D. L. (1934). *Z. vergl. Physiol.* **20**, 617–25.

—— (1940). *J. exp. Biol.* **17**, 267–77.

Mellanby, K. (1939). *Parasitology*, **31**, 200–11.

Necheles, H. (1924). *Pflüg. Arch. ges. Physiol.* **204**, 72–86.

—— (1927). *Chin. J. Physiol.* **1**, 143–56.

THE HUMIDITY BEHAVIOUR OF THE MEALWORM BEETLE, *TENEBRIO MOLITOR* L.

I. THE REACTION TO DIFFERENCES OF HUMIDITY

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(With Four Text-figures)

Humidity is well known to be a factor of great importance in the lives of many insects and other terrestrial arthropods. Apart, however, from the pioneer work of Shelford (1913) and his school on the rather complex stimulus of "evaporating power of the air", there has been, until recently, little detailed and exact study of the behaviour of animals towards humidity as a stimulus. The recently developed methods of Gunn & Kennedy (1936) now make such study possible. By means of these methods, humidity behaviour has been examined in the wood-louse, *Porcellio scaber*, by Gunn (1937); in the locust, *Locusta migratoria*, by Kennedy (1937); in the mosquitoes, *Anopheles gambiae* and *A. funestus*, by De Meillon (1937); in the mosquito, *Culex fatigans*, by Thomson (1938) and in the cockroach, *Blatta orientalis*, by Gunn & Cosway (1938). The work described below deals with the reactions of the adult mealworm beetle, *Tenebrio molitor* L., towards humidity. This beetle lives in flour, bran and similar products and is moderately resistant to desiccation. It loses about 4% of its weight per day in dry air at 25° C. and dies in 8–9 days. Its resistance to desiccation is therefore less than that of its larva, which was investigated by Buxton (1930).

METHODS

The apparatus used in this work was the alternative chamber described by Gunn & Kennedy (1936). In this chamber, the humidity is higher on one side than on the other, and the number of animals observed on each side is noted. The humidities are controlled by sulphuric acid-water mixtures (Wilson, 1921) and the extreme values of the humidity gradient thus set up in the chamber are measured with Edney hygrometers. The animals can be offered a variety of humidity alternatives, varying both in the difference between the upper and lower extremes in a chamber and in their specific values. Thus a difference of 20% relative humidity (R.H.) can be provided either with specific values of 90 and 70% R.H. or with specific values of 30 and 10% R.H.

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[1] Apart from preliminary experiments, all the experimental work was done by D. P. P.

The apparatus was used in the manner described by Gunn (1937), in which five animals are used in one chamber and forty observations made at 15 min. intervals, thus yielding 200 position records in each experiment. Each experiment lasts 10 hr. and extends over 2 days. About a dozen experiments can be carried out concurrently under these conditions. After each observation the animals are activated mechanically, by pushing them with a cotton covered wire (pipe-cleaner). Each set of position records shows the number of animals on the drier and on the moister sides of the chamber; records of animals in a narrow middle zone, which cannot properly be assigned to either side, are put into a separate category.

The intensity of reaction shown is expressed in two ways: (1) as the ratio of position-records on the drier side to those on the wetter side (D/W ratio; Gunn, 1937) and (2) as the excess percentage of records on the drier side $\left(\frac{100\,(D-W)}{D+W}\right)$ (Gunn & Cosway, 1938). In both expressions, the few records for the middle zone are ignored. In the former case, the theoretical value for no reaction at all is 1·0, while in the latter case it is 0·0%. In Table I is shown a set of data from some typical reactions. The data for a particular series of experiments, under conditions which give an intense reaction, are shown in Fig. 3 and will be referred to later.

Table I. *Results obtained from four typical experiments at* 25° C.

Index no. of exp.	A. 29	A. 47	B. 28	D. 3
Relative humidities in the chamber (% R.H.):				
(*a*) Highest	29½	48	85	99½
(*b*) Lowest	9½	11	42	92½
(*c*) Difference (*a*−*b*)	20	37	43	7
Number of records in each zone:				
Drier (*D*)	130	114	160	191
Moister (*W*)	69	76	33	9
Middle	1	10	7	0
Intensity of reaction:				
(1) As ratio *D*/*W*	1·9	1·5	4·9	21·2
(2) As excess percentage	30·5 %	19·0 %	63·5 %	91·0 %

Usually, for each experiment, there was a control in which all four dishes in the chamber contained a single concentration of sulphuric acid, so that the humidity was uniform throughout the chamber. Any collection of animals on one side in such a control was due to chance or to factors other than humidity. The controls corresponding to the experiments of Table I are shown in Table II. In a total of 119 controls, the average mean intensity of reaction was very close to the theoretical expectation ($D/W=0{\cdot}97$ instead of 1·0; an excess percentage of −1·5 instead of 0·0%). Twice the standard deviation of the average mean excess percentage of all controls was ±18·4%, corresponding to a ratio of D/W of 1·45 or of W/D of 0·69. Thus in a single experiment, if the ratio obtained is over 1·45, the probability is at least 21 to 1 that a reaction has taken place.

In these experiments the two sexes were kept separate, having been separated in the pupal stage with the aid of the diagnostic features described by Hein (1920*b*).

Table II. *Results obtained in controls corresponding to the experiments shown in Table I*

Index no. of exp.	A. 29	A. 47	B. 28	D. 3
Relative humidity in the control chamber (uniform throughout)	19½ %	40 %	63½ %	90 %
Number of records in each zone:				
"Drier" (*D*)	96	103	89	110
"Moister" (*W*)	99	95	97	87
Middle	5	2	14	3
"Intensity of reaction":				
(1) As ratio "*D*/*W*"	0·97	1·08	0·90	1·26
(2) As excess percentage	−1·5 %	4·0 %	−4·0 %	11·5 %

This separation was necessary because Valentine (1931) has demonstrated a reaction to sex smell in *Tenebrio molitor*. In order to avoid any chance of desiccation, cultures were kept as moist as possible, consistent with avoiding infestation by mites or fungi; on the day before an experiment, the animals to be used were kept in a saturated atmosphere and provided with water to drink. De Meillon (1937) and Gunn & Cosway (1938) have shown that in some insects the direction of the reaction to humidity (i.e. positive or negative) depends on whether the animals have been desiccated or not; in this work on *Tenebrio* the possibility of desiccation has been excluded. The insects were reared at 25° C., using methods given by Hein (1920*a*) and Buxton (1930).

The experiments were carried out in a constant temperature room at 25 ± 0·5° C. and under constant artificial light; the intensity of the light varied from 22 to 42 metre-candles at different points on the benches.

GENERAL RESULTS

In nearly all the experiments the majority of the animals were seen to be on the drier side, whatever the humidities available; in no case was there a significant excess on the moister side. A typical set of results has been shown in Table I. The results of the whole series of experiments are shown in Fig. 1, in which the ratio *D*/*W* is the measure of the intensity of reaction. The highest relative humidity available in the chamber is plotted against the intensity of reaction; the number near each point indicates the difference between the highest and the lowest humidities available in the chamber in that particular experiment. For instance, if the highest relative humidity was 30% R.H. and the number by the point is 20, then the lowest humidity in that experiment was 10% R.H. The unbroken line indicates the theoretical value for no reaction and the broken line shows the value equal to twice the standard deviation obtained from controls.

From this graph, two things are apparent. First, the intensity of reaction is very clearly determined by the specific value of the highest humidity in the chamber. Second, the extent of the range available—i.e. the difference between the highest nd lowest humidities available, or the steepness of the gradient—is not important n determining the intensity of reaction. When the upper humidity is below o % R.H., the reaction, although significant, is always slight however great the lifference of humidity available; but above 70 % R.H. the reaction increases apidly in intensity and becomes maximal when the highest humidity available

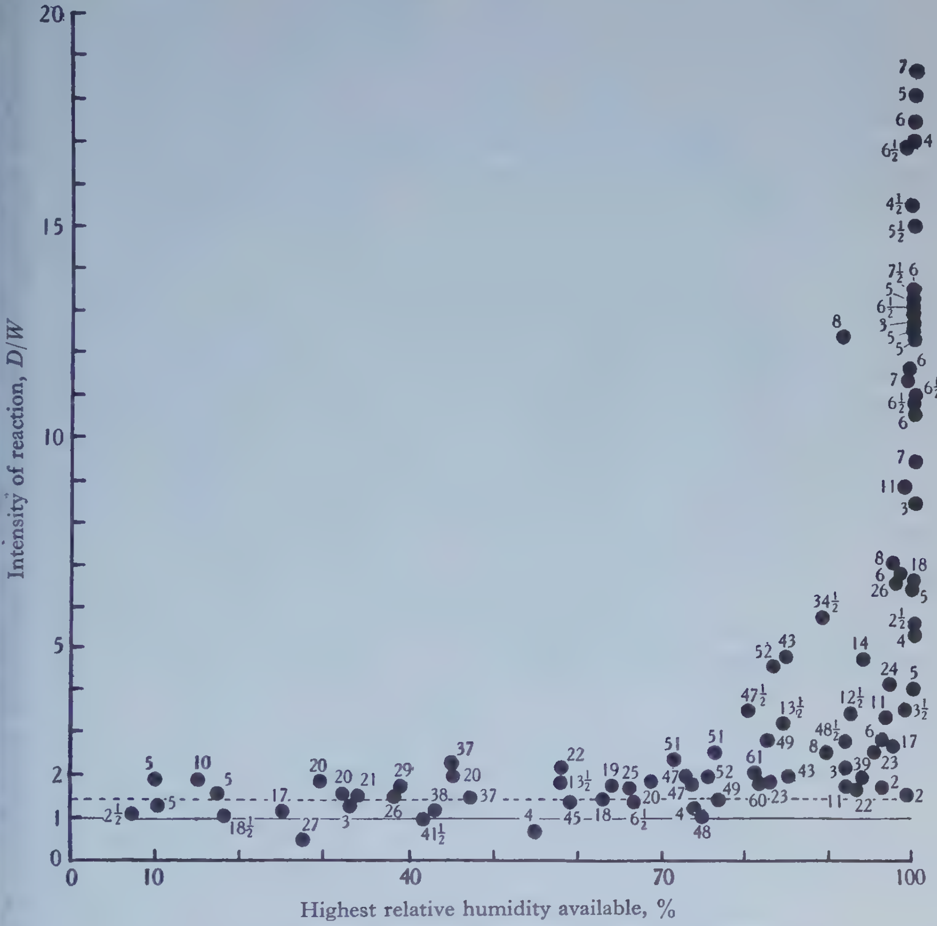
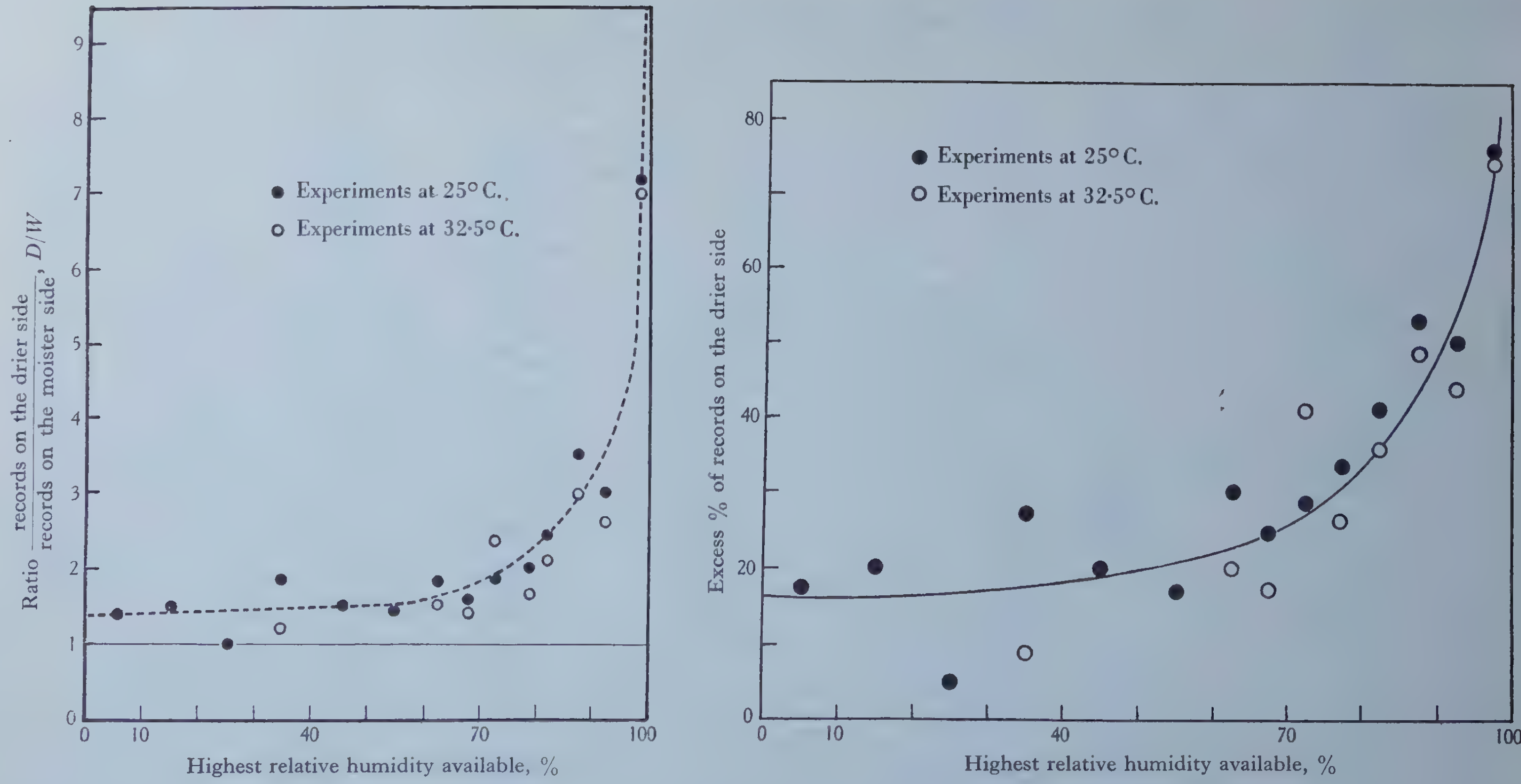


Fig. 1. Results of separate experiments, showing intensity of reaction—expressed as a ratio D/W—developed under various humidity conditions. The figure by each point gives the difference (% R.H.) between the highest and lowest humidities available in the chamber in the corresponding experiment. There is a reaction at all humidities, always towards the dry side, but it becomes intense only when the highest humidity available is very high.

is 100 % R.H. In other words, the higher the value of the highest humidity, the more intensely it is avoided. Since the extent of the range of humidity available in a chamber is of little importance in determining the intensity of reaction, the data of Fig. 1 can be grouped irrespective of range; thus in Fig. 2 the results are grouped in classes of 5 % R.H. above 60 % R.H., where more experiments were done, and in classes of 10 % R.H. below 60 % R.H. Each point indicates the mean intensity of



a

b

Fig. 2. Results shown in Fig. 1 grouped according to the highest humidity available in the chamber and irrespective of the difference of humidity available. *a* intensity of reaction expressed as a ratio D/W. *b*, intensity of reaction expressed as excess percentage on the dry side $\left(\frac{100\,(D-W)}{D+W}\right)$.

reaction for all the experiments in that class, plotted against the mean highest humidity available. Data obtained from some experiments at a higher temperature are also shown in this figure and will be referred to later.

When the data of Fig. 1 are plotted against the lowest humidity available instead of against the highest, there is a considerably greater scatter of points, indicating a less exact correlation.

The reaction increases as the highest humidity available approaches saturation. This is very similar to the results obtained by Thomson (1938) for the mosquito, but is in direct contrast to that of Kennedy (1937) for the locust. Kennedy found that the range available determined the intensity of reaction and that the specific values of the humidities were unimportant. Gunn (1937) found both factors of importance in the wood-louse.

THE INTENSE REACTION

The intense reaction at the moist end of the humidity scale can be made use of in investigating the location of humidity receptors (Pielou, 1940) and the mechanism of the humidity reaction (Gunn & Pielou, 1940). It was therefore examined in greater detail and was found to be very consistent as well as very intense. A standard gradient was adopted in which the controlling fluids were pure water and a sulphuric acid solution with an equilibrium vapour pressure giving a relative humidity of 90% at 25° C. (18·5% sulphuric acid by weight at 25° C.; Wilson, 1921). These fluids gave a gradient extending from very nearly 100% R.H. down to 94% R.H. in the standard 22 cm. chamber used in this laboratory. In twenty-nine experiments involving 145 animals and giving 5800 position records, 90·2% (S.D. 4·35%) of the position records were from the drier side at 94–97% R.H., 6·1% (S.D. 2·45%) from the moist side at 97–100% R.H., and the rest (3·7%) from the middle zone. This gives the high excess percentage on the drier side of 84·1% (i.e. 90·2 − 6·1), corresponding to a *D/W* ratio of 14·8. These data are shown graphically in Fig. 3.

In a series of six experiments in which the development of this reaction was recorded quantitatively, the reaction started very rapidly and increased progressively more slowly until after 15 min. it had practically developed its maximum value (Fig. 4).

EFFECT OF TEMPERATURE ON THE REACTION

A series of experiments, covering the entire range of humidity, was carried out in a constant temperature room at 32·5 ± 0·5° C. The results obtained were almost identical with those in Fig. 1 and the data are grouped and shown in Fig. 2, along with the results of experiments at 25° C. The very close similarity of the two sets of points suggests that the animals react to relative humidity rather than to saturation deficiency. When the data for the two temperatures are plotted against saturation deficiency instead of relative humidity, there is a fairly marked distinction between the two sets of points, thus showing a less exact correlation. Thomson (1938) found that the results for *Culex fatigans* also showed a closer correlation with relative humidity than with saturation deficiency. This conclusion is interesting in view of the stress that has been laid on the importance of using saturation deficiency instead of relative humidity in physiological work on the evaporation of water from insects.

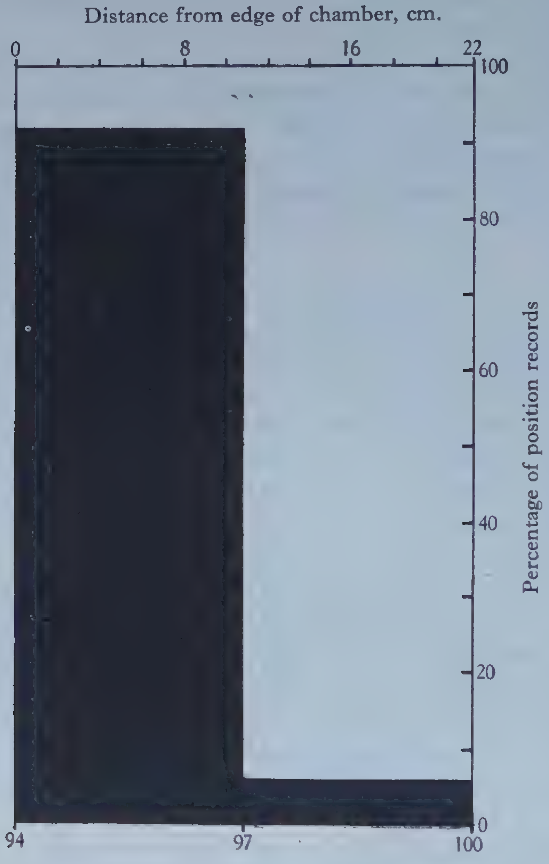


Fig. 3. Showing the marked reaction of *Tenebrio molitor* in an alternative chamber with a humidity gradient extending from 94 to 100% R.H. (*standard gradient*).

An attempt was made to perform a third series of experiments at a lower temperature (14° C.). At this temperature, however, the animals (which had been reared at 25° C.) were very inactive, very slow when they did move, and stiff and awkward in their movements; but they did, nevertheless, show the same general trend of results. The reaction was most intense when a very high humidity was available, but it was not nearly as intense as at higher temperatures. The efficiency of locomotion of these animals was obviously impaired by the cold, so this series of experiments was discontinued.

EXPERIMENTS ON SINGLE ANIMALS

In the experiments described above, five animals were used at a time and their istribution was noted periodically, 15 min. after activating them mechanically. nother series was carried out, using one animal at a time in the standard gradient. ere the movements of the animal were recorded on squared paper and, with the id of an audible time signal, half-minutes were marked on the tracks. From these rawings, the time spent in each half of the chamber was found. Altogether twenty-two such experiments were carried out, covering slightly over 25½ hr. Of this time, 24 hr. 32 min. was spent by the animals in the drier side and in 1 hr. 2 min. in the moister side. This gives an excess percentage on the dry side (time, not position records) of 92%, compared with 84% in the main experiments. In these experiments, the animals were not activated mechanically every 15 min. as they were in the main experiments. The slightly more intense reaction shown is, presumably, partly due to some slight increase in intensity after the first 15 min. (cf. Fig. 4).

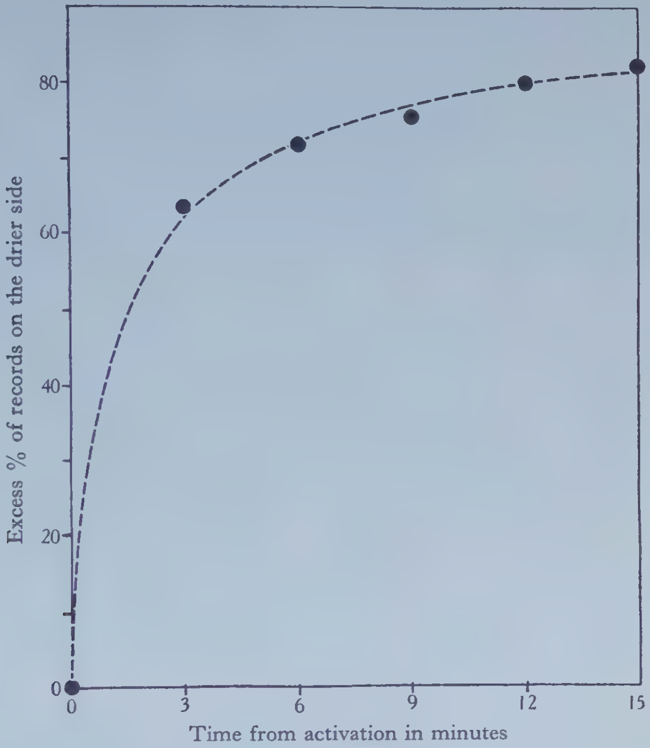


Fig. 4. The development of the reaction in the standard gradient. The reaction is practically fully developed after 15 min.

SUMMARY

1. *Tenebrio molitor* adults show a behaviour reaction to humidity. When normal undesiccated specimens are offered a range of humidities, they tend to collect in the drier region.

2. When the highest relative humidity available is not above 70% R.H., the reaction is slight but real. In a series of tests, as the highest humidity available approaches saturation, the intensity of reaction increases rapidly. It becomes nearly as intense as possible when the moistest region in the gradient approaches 100% R.H.

3. The difference between the two extreme humidities available—or the steepness of the gradient of humidity—is unimportant in determining the intensity of reaction. It is the value of the highest humidity that matters.

4. The intense reaction shown when the upper humidity approaches saturation is very consistent; it reaches its maximum value after about 15 min.

5. The behaviour of these insects appears to be more closely correlated with relative humidity than with saturation deficiency.

REFERENCES

BUXTON, P. A. (1930). *Proc. roy. Soc.* B, **106**, 560–77.
DE MEILLON, B. (1937). *Publ. S. Afr. Inst. med. Res.* **7**, 313–27.
GUNN, D. L. (1937). *J. exp. Biol.* **14**, 178–86.
GUNN, D. L. & COSWAY, C. A. (1938). *J. exp. Biol.* **15**, 555–63.
GUNN, D. L. & KENNEDY, J. S. (1936). *J. exp. Biol.* **13**, 450–9.
GUNN, D. L. & PIELOU, D. P. (1940). *J. exp. Biol.* **17**, 307–16.
HEIN, S. A. A. (1920*a*). *Verh. Akad. Wet. Amst.* **23**, 193–218.
—— (1920*b*). *J. Genet.* **10**, 227–64.
KENNEDY, J. S. (1937). *J. exp. Biol.* **14**, 187–97.
PIELOU, D. P. (1940). *J. exp. Biol.* **17**, 295–306.
SHELFORD, V. E. (1913). *Biol. Bull. Wood's Hole*, **25**, 79–120.
THOMSON, R. C. M. (1938). *Bull. ent. Res.* **29**, 125–40.
VALENTINE, J. M. (1931). *J. exp. Zool.* **58**, 165–228.
WILSON, R. E. (1921). *J. industr. Engng Chem.* **13**, 326–31.

THE HUMIDITY BEHAVIOUR OF THE MEALWORM BEETLE, *TENEBRIO MOLITOR* L.

II. THE HUMIDITY RECEPTORS

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(*Received* 23 *February* 1940)

(With Seven Text-figures)

The existence of sensillae sensitive to humidity has frequently been surmised, but there is no experimental evidence on their form or position in insects. Necheles (1925) suggested that the antennal hairs of the mosquito might be sensitive to humidity; Thomson (1938) made an attempt to locate the receptors in the mosquito, *Culex fatigans*, but found the experimental difficulties too great. Kennedy (1937) attempted unsuccessfully to locate the receptors in the locust, *Locusta migratoria*. Gunn & Cosway (1938) found that humidity behaviour in the cockroach, *Blatta orientalis*, became irregular when the antennae were infected with a fungus, and they suggested on these grounds that the receptors are located on the antennae. Thus there is no satisfactory evidence on the site of humidity receptors in insects. The problem has also been examined in other terrestrial arthropods. Blumenthal (1935), working on the olfactory sense of spiders, decided that certain organs on the tarsi of the legs are olfactory receptors. As he used water as one of his "odours" and found a reaction to it, he concluded that these organs were also humidity receptors. Gunn (1937) could not locate the humidity receptors in the wood-louse, *Porcellio scaber*.

In the work described here, the humidity receptors of the beetle, *Tenebrio molitor* L., have been located, and an attempt has been made to identify them. It has already been shown that this insect reacts clearly to humidity (Pielou & Gunn, 1940); the general methods used for that work proved suitable for the work described here.

LOCALIZATION OF RECEPTORS

Before the receptors can be identified, they must be located by amputation, covering with wax or similar methods. In order to facilitate such treatment, insects to be operated upon were first cooled to a temperature low enough to make them inactive; cooling was found to be more satisfactory than the use of anaesthetics. This procedure was found to have no ill effects, for after recovery unoperated animals gave the usual humidity reactions described by Pielou & Gunn (1940).

Fortunately the first organs to be examined were the antennae and maxillary palps, and the experiments at once revealed the site of the receptors.

Tests were carried out using the standard gradient (Pielou & Gunn, 1940, p. 291), in which animals are exposed to a short gradient of humidity from 94 to 100% relative humidity (% R.H.). Under these conditions, normal animals show an intense reaction, collecting on the drier side of the chamber at 94–97% R.H. and avoiding the moister side at 97–100% R.H.

The antennae of animals to be tested were amputated with scissor-forceps in such a way as to leave only the basal segment intact; that is, ten of the eleven antennal segments were removed. Eight experiments involving 40 animals were carried out on beetles treated in this way and controls with normal animals were carried out concurrently. The amputation of the antennae completely abolished the reaction to humidity and there was no recovery even after several weeks.

A similar series of experiments was carried out in which the maxillary palps instead of the antennae were amputated on both sides. Animals treated in this way gave just as intense a reaction as normal animals. It seems, therefore, that the sense organs concerned are confined to the antennae and do not occur on the maxillary palps.

It might be thought that sense organs may be present on the maxillary palps, perhaps in numbers too small to affect the intensity of reaction. This criticism is dealt with later, but it may be pointed out here that elimination of a single segment of the antenna has a marked effect on the intensity of reaction, while removal of both maxillary palps has none.

Animals without antennae behaved in a normal manner, apart from their humidity behaviour; so did those from which the palps had been removed. The insects suffered no obvious ill effects in either case.

ACTIVITY OF ANTENNA-LESS *TENEBRIO*

Experiments involving the amputation of antennae have been criticized because abnormally high or low activity may result from the operation, thus causing an apparent abolition of sensitivity to a particular stimulus, and so leading to the unjustified conclusion that the receptors concerned are located on the antennae. Several workers, including von Frisch (1921), have shown that amputation of antennae need not affect reactions mediated by receptors located elsewhere. Marshall (1935), in an extensive review of the olfactory function of the insect antenna, also came to the conclusion that amputation was a legitimate means of elimination. There is some evidence, however, that the antennae of some insects bear "stimulatory organs" (Wolsky, 1933) which affect general activity quite apart from specific stimulation. In the bug, *Rhodnius prolixus*, for example, Wigglesworth & Gillett (1934) found that if the antennae are removed the insect falls into a state of "akinesis" or inactivity from which it is not easily aroused. It was therefore necessary to examine the activity of antenna-less beetles.

The activity of normal beetles kept at various constant relative humidities is described quantitatively by Gunn & Pielou (1940, Fig. 3). Precisely similar experiments were carried out with antenna-less animals. The differences of locomotory activity shown by normal animals kept at different humidities were completely abolished (Fig. 1), but the resultant activity level was neither very low nor very high; it was below that shown by normal animals kept at 100% R.H. and above that shown by normal animals kept at 0% R.H.

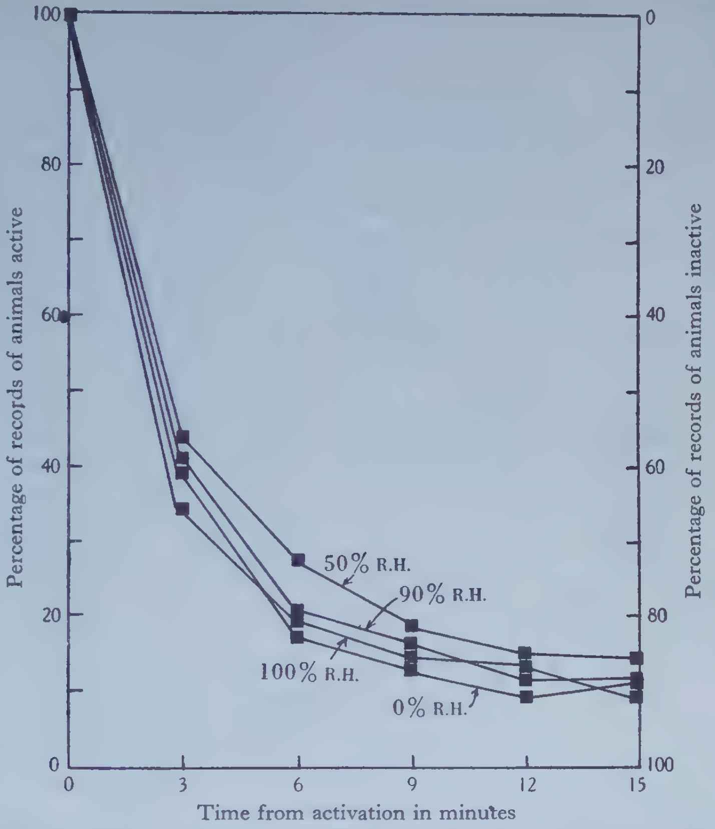


Fig. 1. Decline in activity with time in *Tenebrio molitor* deprived of their antennae and kept at various uniform humidities. The humidity does not affect activity, as it does with animals which have their antennae (see Fig. 3, Gunn & Pielou, 1940), and the activity is not depressed or increased outside the normal range by the amputation.

It is therefore clear that amputation of the antennae does not have any general ill effect on the mealworm beetle and that the antennae have no considerable function as "stimulatory organs".

THE SENSE ORGANS ON THE ANTENNAE AND ON THE MAXILLARY PALPS

A morphological examination of the antennae and palps was made by Valentine (1931), who gave a description of the various sensillae; he did not give the number of each kind of sensilla. In order to get this information, whole mounts

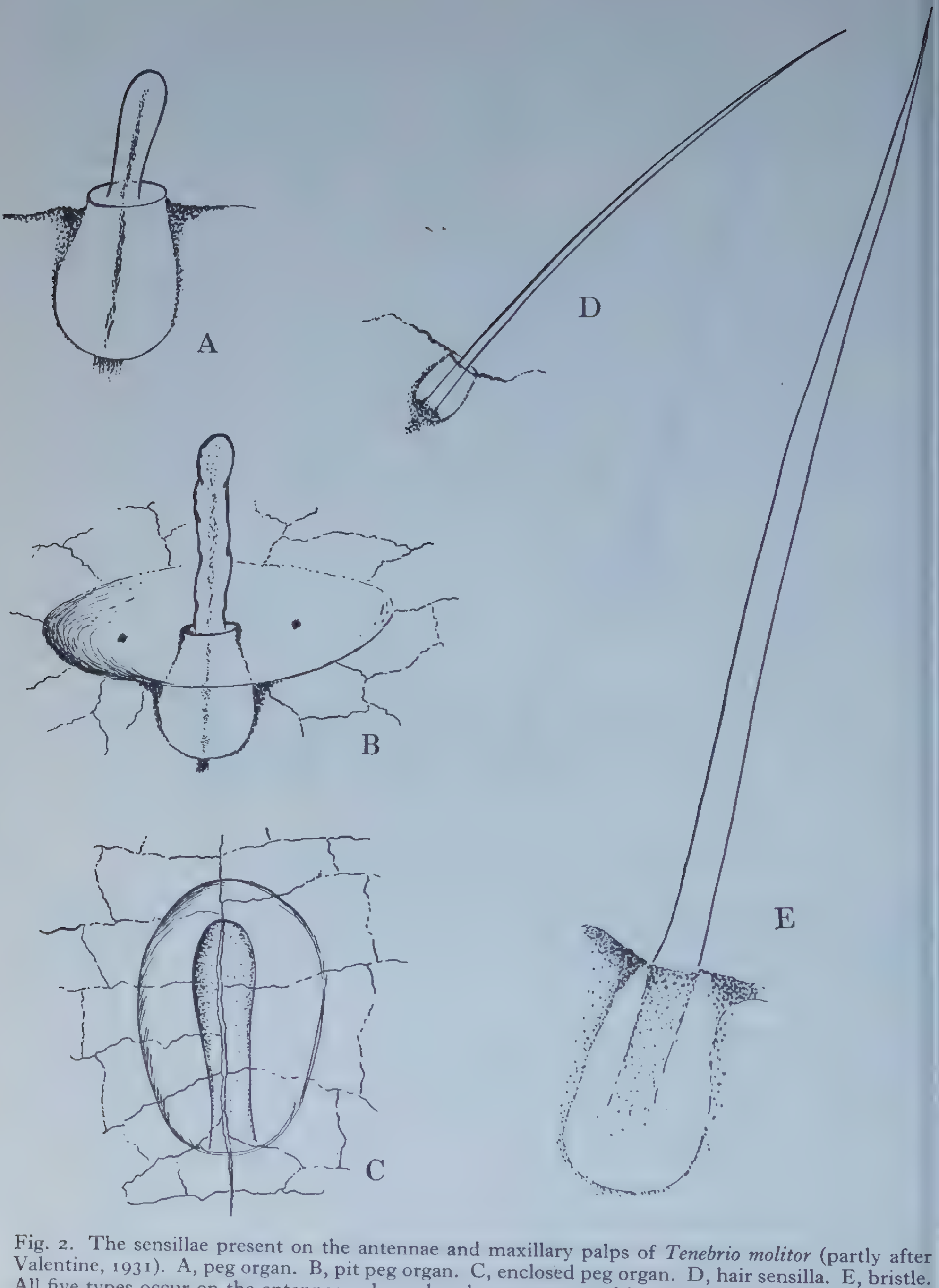
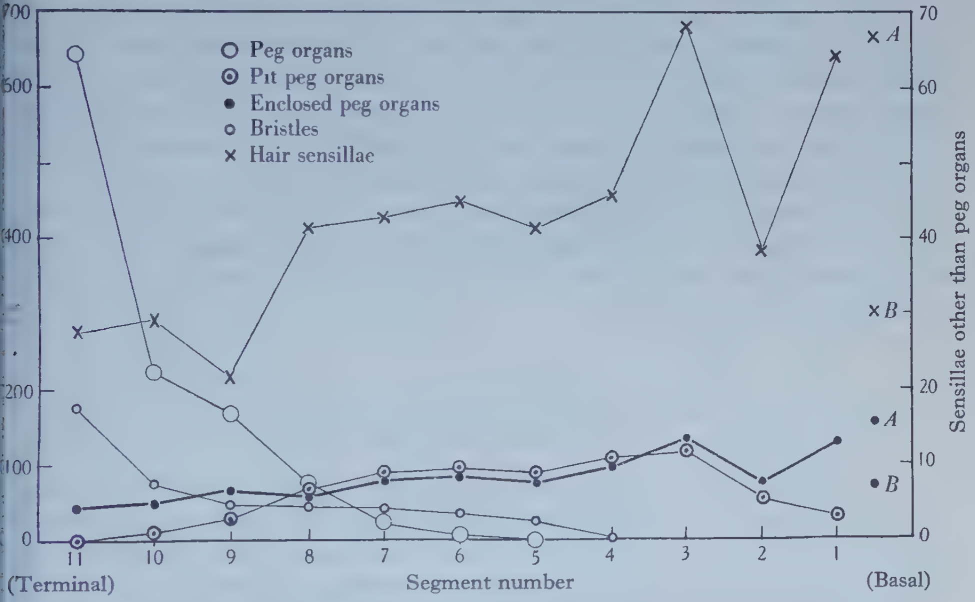


Fig. 2. The sensillae present on the antennae and maxillary palps of *Tenebrio molitor* (partly after Valentine, 1931). A, peg organ. B, pit peg organ. C, enclosed peg organ. D, hair sensilla. E, bristle. All five types occur on the antenna; only enclosed peg organs and hair sensillae occur on the palps. Hair sensillae and bristles are drawn on a smaller scale than the others.

these appendages were made, the material being obtained from young adults hich had just emerged from the pupae. The cuticle of these newly emerged adults not yet blackened and is softer than in older animals, but all the sense organs e fully formed.

The antenna consists of eleven segments, some very slightly longer than hers, but all of approximately the same form. The structures found on the tennae are described below and illustrated in Fig. 2.

(1) *Peg organs* (Basiconic sensillae). These are thin-walled pegs, the bases of hich are expanded into a distinct basal bulb. They are by far the most numerous gans on the antenna and are very densely crowded on the terminal (distal) gment (Fig. 2A).



g. 3. Numbers of each type of sensilla on the eleven segments of the antenna of *Tenebrio molitor*. he numbers of hair sensillae and enclosed peg organs on the terminal (A) and subterminal (B) gments of the maxillary palps are also shown. N.B. Scale for peg organs on the left, scale for her sensillae on the right.

(2) *Pit peg organs* (Coeloconic sensillae). These are peg organs set in a bowl-like epression, the pit. The pegs are longer than those of ordinary peg organs. The pit" is much wider and much shallower than in most coeloconic sensillae ig. 2B).

(3) *Enclosed peg organs* (Ampullaceous sensillae). Valentine (1931) describes ese as peg organs set in a flask-like cavity which communicates with the exterior y means of a slit. I was able to see the slit in a few cases only; the organ generally ad the appearance of being quite enclosed and cut off from the exterior (Fig. 2C).

(4) *Hair sensillae.* These are thin-walled setiform sensillae (Fig. 2D).

(5) *Bristles or spines.* These are thick-walled, long, stiff structures, much igger than and quite distinct from the hair sensillae (Fig. 2E).

The first four of these kinds of sensillae belong to types which are usuall described as chemo-receptors (Snodgrass, 1926; Eltringham, 1933; Wigglesworth 1939). The bristles, on the other hand, are usually described as tactile, and th thickness of their walls makes it unlikely that they are chemo-receptors of any kind

Two of these five types of sensilla—the enclosed peg organs and the hai sensillae—are found on the maxillary palps, where they are indistinguishable i form from those on the antennae. Since amputation of the palps does not affec the reaction to humidity, it is unlikely that they carry any humidity receptors, s the enclosed peg organs and the hair sensillae can be eliminated. None of the fiv types of organ occurs in identical form elsewhere on the body.

The quantitative distribution of these sensillae was obtained by counting them on each segment of twenty-four antennae, mounted whole. The results are show in Fig. 3, the points representing averages from the twenty-four antennae. A muc smaller scale has been used for peg organs because of their very large number No significant difference was found in the numbers for the two sexes. In Fig. the numbers of enclosed peg organs and hair sensillae on the terminal (A) and sub terminal (B) segments of the maxillary palps are also shown.

It will be seen that the peg organs are confined to the distal six segments of th antenna, and the bristles to the distal seven segments, while pit peg organs occur o each segment except the terminal one and enclosed peg organs and hair sensilla are present on every segment.

INTENSITY OF REACTION WITH PROGRESSIVE AMPUTATION OF ANTENNAL SEGMENTS

Various numbers of segments of the antennae were cut off, equal numbers fro the two antennae, in the manner already described. The intensity of reaction wa measured in the usual way, using the standard gradient chamber (Pielou & Gunn 1940). The results are shown in Fig. 4, where each point is the mean o 20 experiments (100 animals, 4000 position records). The line drawn on each sid of each point represents twice the standard error of the mean. The measure o intensity of reaction is here the excess percentage of records for the drier sid $\left(\frac{100\,(D-W)}{D+W}\right)$ (Pielou & Gunn, 1940).

From Fig. 4 it appears that there is a significant reaction still remaining whe seven pairs of segments have been removed from the antennae, but not when eigh pairs have been removed. With seven pairs of segments removed (four pairs lef the peg organs and the bristles are all gone (Fig. 5), so neither of these can be th sole humidity receptors, while with eight pairs of segments removed (three pai left) there are still pit peg organs present; there are also hair sensillae and enclose peg organs left, but they also occur on the maxillary palps and have already bee eliminated for that reason. It was found that amputation of both palps did n diminish the intensity of reaction either with antennae intact or with the reactio already reduced by partial amputation of the antennae.

Consequently, we are driven to conclude that the pit peg organs are hygro-eceptive. These sensillae occur not only on the fourth segment but also on the rst three (Fig. 5), but when only three segments remain on each side there is evertheless no reaction to humidity. This suggests that there may have to be a certain threshold number of sensillae present before a reaction can occur, and the number on the three basal segments may be too low. This possibility was investigated by means of asymmetrical amputations (see below). Even so, pit peg organs cannot be the sole hygro-receptors, for while there are none of these sensillae on the eleventh segment, amputation of this segment reduces the intensity of reaction considerably. This question is discussed below.

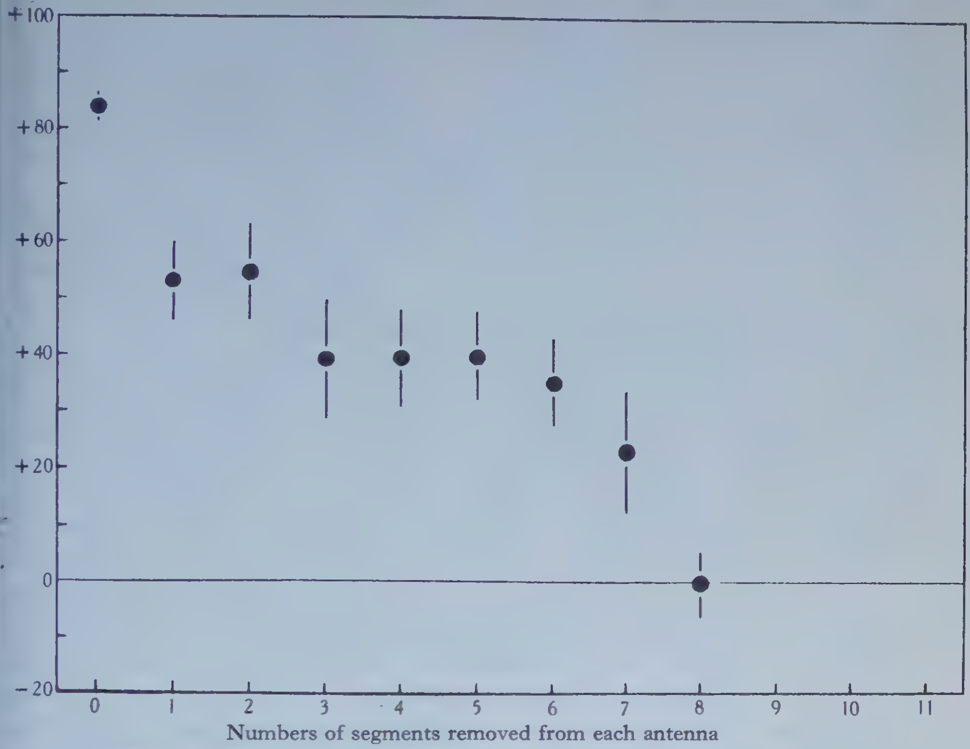


Fig. 4. Intensity of reaction (excess percentage), in the standard gradient of humidity, shown by *Tenebrio* with various numbers of antennal segments amputated symmetrically. Each point represents the mean of twenty experiments and the vertical line represents twice the standard error of the mean on either side. There is a reaction with seven pairs of segments removed but none with eight pairs removed.

ASYMMETRICAL AMPUTATION OF ANTENNAL SEGMENTS

These experiments were carried out in exactly the same manner as those nvolving symmetrical amputations. Amputation of the whole of one antenna, eaving the other intact, did not cause circus movements (Gunn & Pielou, 1940).

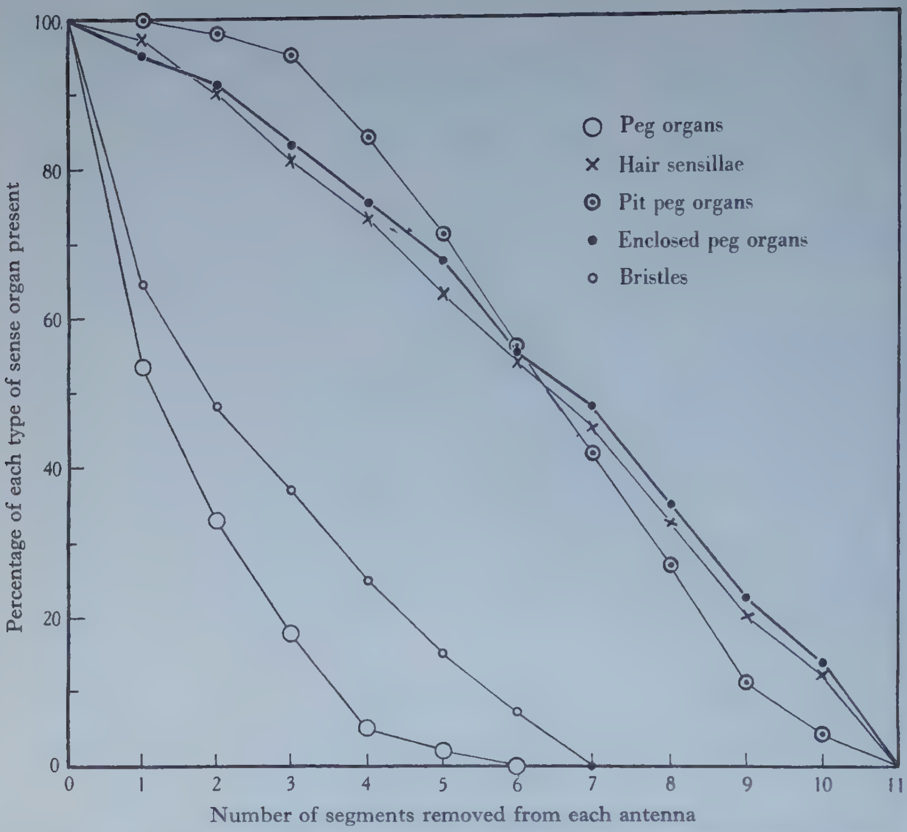
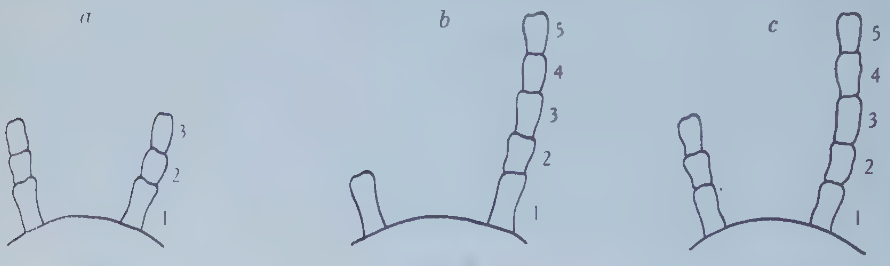


Fig. 5. Proportion of each type of sensilla remaining as the antennal segments of *Tenebrio* are progressively amputated symmetrically. There is still a reaction to humidity when seven segments have been removed, but not when eight have been cut off.



0·0 % (S.E. = ± 1·8 %)

11·0 % (S.E. = ± 2·1 %)

20·1 % (S.E. = 1·9 %)

Fig. 6. Diagrams to show the numbers of antennal segments remaining in the experiments demonstrating the occurrence of humidity receptors on the basal segments, and the results of those experiments. Thus, in the experiment represented by *b*, the intensity of reaction was 11 % (S.E. ± 2 %).

he results are expressed diagrammatically (Fig. 6). Fig. 6*a* shows that with ree segments remaining on each side (total of six segments remaining) there is no action to humidity. Fig. 6*b* shows that when six segments remain asymmetrically -five on one side and one on the other—a significant reaction is obtained. This is uite in keeping with the previous results, since we know from them that receptors e present on segments 4 and 5, but perhaps absent from segments 2 and 3. The ucial experiment is shown in Fig. 6*c*. If segments 2 and 3 bear no receptors for umidity, animals with three segments left on one side and five on the other should how the same intensity of reaction as animals with one left on one side and five n the other, as in Fig. 6*b*. In fact, however, the presence of the segments 2 and 3 oes lead to a significantly more intense reaction. Humidity receptors are therefore ndoubtedly present on these segments, although experiments after symmetrical mputation did not reveal them.

These experiments remove the objection to regarding the pit peg organs as ygro-receptive. Unfortunately for simplicity, these organs diminish in frequency nteriorly and there are none at all on the distal segment. The pit peg organs annot therefore be the *sole* humidity receptors. In the distal region of the antennae, he only other possible structures are the peg organs—which are very similar to it peg organs—and the bristles, which are thick-walled structures most probably actile and protective in function and unlikely to be humidity receptors. As the istribution of these two types is very similar in *Tenebrio molitor*, however, it is ot possible to devise crucial experiments to decide between them with certainty, ut it may be possible in some other species.

Further experiments involving the asymmetrical amputation of the most distal egments made it quite certain that the presence of the terminal segment, on which here is the most dense concentration of peg organs, is very important in determining he intensity of reaction.

THE PROBABLE RECEPTOR STRUCTURES

From the lines of evidence presented above, it appears that the sensillae sensitive o humidity are the pit peg organs and the peg organs. These are both thin-walled ensillae of the type which in general appears to be chemo-receptive; in *Tenebrio olitor* Valentine (1931) identified the peg organs as olfactory receptors. The two unctions are not necessarily inconsistent with one another. The slight possibility hat the thick-walled bristles are hygro-receptive as well as or instead of the peg rgans has not been excluded experimentally.

POSSIBLE MODE OF ACTION OF HUMIDITY RECEPTORS

A receptor sensitive to humidity may function in the same way as an olfactory eceptor, the water vapour being regarded as an "odour", although this is rather egging the question, for very little is known of the mechanism of olfactory eceptors. This mode of action is implied by Blumenthal (1935). It is more likely that a humidity receptor acts like a hygrometer. For instance, the receptor ma act like an atmometer, evaporation of water taking place at a rate which depend on the dryness of the air and resulting in quantitative changes of chemical concentration, osmotic pressure or mechanical stress in the receptor cells. Again, th receptor might act like a wet bulb thermometer, evaporation resulting in a lowerin of temperature in special cells and being detected by heat receptors. In instrument working on any of these principles, saturation deficiency or the evaporating powe

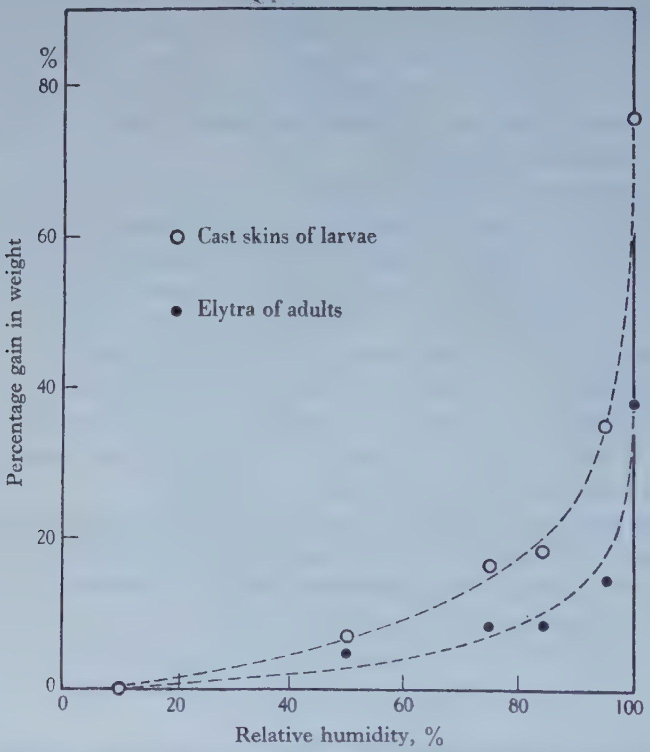


Fig. 7. Hygroscopic properties of samples of dead cuticle from *Tenebrio molitor*. The material wa kept at each humidity until constant in weight. The weights are expressed as gains over the weigh at 10% R.H.

of the air would be a more suitable measure of humidity than relative humidity relative humidity could be estimated only when additional factors had been assessed.

It has been shown, however, that the reaction of *Tenebrio* is more closely correlated with relative humidity than with saturation deficiency (Pielou & Gunn 1940). The only hygrometers recording relative humidity directly depend on the hygroscopic properties of materials like hair and paper, and Necheles (1925) and Thomson (1938) have suggested that mosquitoes have hygro-receptors working on this principle. The hygroscopic properties of dead cuticle of *Tenebrio* were therefore examined to see if this material is suitable for hygrometry. The parts chosen were e elytra of adults and the cast skins of larvae. The material was kept at constant umidity until its weight had become constant, it was then transferred to another umidity and weighed, and so on. Fig. 7 shows the results. It will be seen that the ticle is markedly hygroscopic, and that its weight is most sensitive to humidity hanges in the upper part of the humidity scale. For a 10% change of relative umidity, the weight changes 20–40 times as much above 90% R.H. as it does elow 80% R.H. These curves are strikingly similar to those relating the intensity reaction of *Tenebrio* to the relative humidity (Pielou & Gunn, 1940, Figs. 1, 2).

The dead cuticle comes very slowly to its equilibrium weight (some hours to e day), while the thin-walled hygro-receptors (pegs) must be supposed to do so ery quickly, for the behaviour reaction approaches completion in about 15 min. ielou & Gunn, 1940). The main difficulty arises, however, in trying to imagine ow the hygrometrically effective part of the cuticle can be more affected by the umidity of the atmosphere than by the water inside the body.

SUMMARY

1. Removal of both antennae of the mealworm beetle, *Tenebrio molitor*, com- letely abolishes the animal's reaction to humidity. Removal of both maxillary alps has no effect on the reaction.

2. The quantitative distribution of the five types of sensillae present along the even segments of the antenna is described. Two of these types occur also on the axillary palps.

3. Progressive symmetrical amputation of antennal segments leads to a gradual duction of the humidity reaction to zero; there is a reaction with four segments maining on each side and no reaction when only three segments remain.

4. Asymmetrical amputation of the more basal segments shows that humidity eceptors are nevertheless present on these and that a threshold number of sensillae ust be left in action before a reaction occurs.

5. The experimental work, taken in combination with the observations in the istribution of sensillae, shows that the pit peg organs are hygro-receptive. Either he peg organs or the bristles or both are hygro-receptive as well. It is, however, nlikely that the bristles are hygro-receptive.

6. Experimental work on the locomotory activity of this beetle shows that the onclusions are not invalidated by any general ill effects of amputation of antennae.

7. It is suggested that the hygro-receptors function hygroscopically.

REFERENCES

Blumenthal, H. (1935). *Z. Morph. Ökol. Tiere*, **29**, 667–719.
Eltringham, H. (1933). *The Senses of Insects*. London.
Frisch, K. von (1921). *Zool. Jb.* Abt. allg. Zool. Physiol. **38**, 449–514.
Gunn, D. L. (1937). *J. exp. Biol.* **14**, 178–86.
Gunn, D. L. & Cosway, C. A. (1938). *J. exp. Biol.* **15**, 555–63.
Gunn, D. L. & Pielou, D. P. (1940). *J. exp. Biol.* **17**, 307–316.

KENNEDY, J. S. (1937). *J. exp. Biol.* **14**, 187–97.
MARSHALL, J. (1935). *Trans. R. ent. Soc. Lond.* **83**, 49–72.
NECHELES, H. (1925). *Arch. Schiffs.- u. Tropenhyg.* **29**, 288–91.
PIELOU, D. P. & GUNN, D. L. (1940). *J. exp. Biol.* **17**, 286–94.
SNODGRASS, R. E. (1926). *Smithson. misc. Coll.* **77**, 1–80.
THOMSON, R. C. M. (1938). *Bull. ent. Res.* **29**, 125–40.
VALENTINE, J. M. (1931). *J. exp. Zool.* **58**, 165–228.
WIGGLESWORTH, V. B. (1939). *Principles of Insect Physiology.* London.
WIGGLESWORTH, V. B. & GILLETT, J. D. (1934). *J. exp. Biol.* **11**, 120–39, 408.
WOLSKY, A. (1933). *Biol. Rev.* **8**, 370–417.

THE HUMIDITY BEHAVIOUR OF THE MEALWORM BEETLE, *TENEBRIO MOLITOR* L.

III. THE MECHANISM OF THE REACTION

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(*Received* 21 *April* 1940)

(With Four Text-figures)

T has previously been shown that, under suitable experimental conditions, *Tenebrio nolitor* reacts clearly to humidity (Pielou & Gunn, 1940) and that the humidity eceptors are probably the pit-peg organs and peg organs on the antennae (Pielou, 940). The present work is an attempt to analyse the mechanism of this reaction oth qualitatively and quantitatively, in terms of elementary animal behaviour.

The analysis of the mechanism of behaviour reactions of animals to diffuse nolecular stimuli—odours, humidity and air temperature (as distinct from "radiant heat")—has been attempted only on a limited scale. This is no doubt due o the difficulties of controlling and measuring the conditions and of evolving a echnique as accurate and precise as that which can be used when light is the timulus. Such analyses as have been made, in general, attempt to carry over the heories and classifications—especially the classification of Kühn (1919)—that ave been developed mainly in relation to light. The modifications made by Gunn Fraenkel & Gunn, 1940) in Kühn's classification make it more suitable for the nclusion of reactions to diffuse stimuli.

This system includes two main categories: *kineses*, or undirected reactions, in vhich the direction of movement of the animals is not precisely related to the lirection of the source of stimulation, and *taxes*, the directed reactions in which he orientation of the axis of the animal's body is influenced by the direction of the ource. In *ortho-kinesis*, the average velocity of locomotion or the frequency of ctivity depends on the intensity of stimulation, and aggregation occurs in the egion of lowest velocity; in *klino-kinesis* (avoiding reactions) the amount of turning luring locomotion depends on the intensity of stimulation and upon some other actor like sensory adaptation (Ullyott, 1936; Gunn, *et al.* 1937). In *klino-taxis* the nimal compares the intensities of stimulation in its vicinity by alternative movenents to the left and right (either movements of the whole body or of some receptor egion such as its antennae) and the comparison is thus between two intensities vhich are successive in time. In *tropo-taxis* the behaviour is similar but orientation is immediate, by simultaneous comparisons of intensities at two symmetricall placed receptors. *Telo-taxis* seems to be out of the question with diffuse stimul (Fraenkel & Gunn, 1940).

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[1] All the experimental work was done by D. P. P.

There have been several reports of directed reactions (taxes) to odours, fo example by Barrows (1907) and Flügge (1934) on *Drosophila*, by Warnke (1931) o *Geotrupes*, by Murr (1930) on *Habrobracon* and by Hartung (1935) on *Calliphora* Fraenkel's analysis of these reports (Fraenkel & Gunn, 1940) suggests that a whol series of reactions (ortho-kinesis, klino-kinesis, klino-taxis and tropo-taxis) com into action in turn as the animal approaches the source of stimulation and get into a steeper and steeper gradient. No directed reaction to the stimulus of humidity has yet been described but ortho-kinesis towards humidity has been established fo the woodlouse (Gunn, 1937) and for the locust (Kennedy, 1937).

The material, apparatus and methods used in this work have been described in the main, in previous papers (Gunn & Kennedy, 1936; Gunn, 1937; Pielou & Gunn, 1940; Pielou, 1940).

DIFFERENTIAL ACTIVITY IN THE ALTERNATIVE CHAMBER

Experiments were carried out with the humidity alternative chamber (Gunn & Kennedy, 1936) using the short gradient of humidity from 94 to 100% R.H. i which *Tenebrio molitor* shows a very intense reaction (Pielou & Gunn, 1940) Here the animals were used one at a time and the paths of the animals in the chambe were followed and recorded on squared paper. The false floor of the chamber wa marked off into squares to facilitate this recording. A mechanical device sounding at half-minute intervals was used and time marks were recorded on the tracks this allowed the rates of movement and times spent on each side of the chamber t be worked out. Twenty-two animals were observed in this way for a total period o slightly over 25½ hr.

The times spent on the drier side (94—97% R.H.) and on the moister side (97—100% R.H.) were obtained from these track records. The detailed results are shown in Table I and their most striking points shown in Fig. 1. The total time spent on the drier side is, of course, far greater than that spent on the moister side, and this is in keeping with previous experiments conducted in a different manne (Pielou & Gunn, 1940). It is also seen from Table I and Fig. 1 that the animals practically never come to rest on the moister side, they remain continuously active there, only 6% of their total time on this side being spent motionless. On the drie side, however, over 80% of their time is spent inactive and, as the total time spent on the drier side is greater than on the moister, the ratio of the absolute times spent in the inactive state on the two sides is even more striking (19½ hr. to 4 min.).

This very marked differential activity observed with such a small difference of humidity undoubtedly accounts for the very intense reaction to a great extent. A similar pronounced hygro-kinesis, but in the reverse direction, was found in the woodlouse, *Porcellio scaber*, by Gunn (1937). On the other hand in the mosquito,

Table I. *Times spent by* Tenebrio *on the drier and moister sides of the standard humidity gradient alternative chamber. Total times from twenty-two experiments*

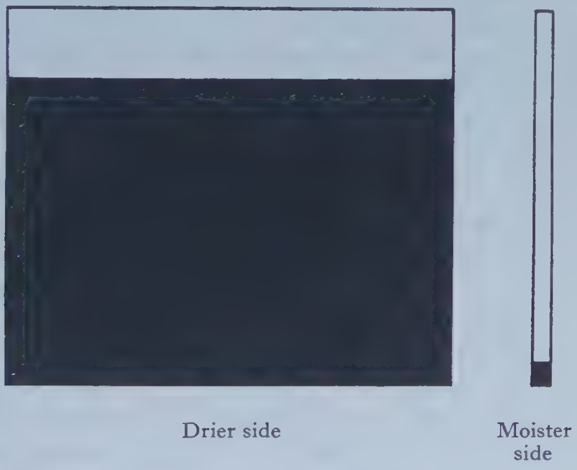
	Drier side 94–97% R.H.	Moister side 97–100% R.H.
Total time spent on each side	24 hr. 32 min.	1 hr. 2 min.
Time spent *inactive*	19 hr. 38 min.	0 hr. 4 min.
Time spent *active*	4 hr. 54 min.	0 hr. 58 min.
% time spent *inactive*	80·0	6·5
% time spent *active*	20·0	93·5

Ratios of times spent on the two sides $\frac{\text{Drier side}}{\text{Moister side}}$

(1) Total times $=\frac{24\text{ hr. }32\text{ min.}}{1\text{ hr. }\ 2\text{ min.}}=23{\cdot}7$

(2) Times spent *inactive* $=\frac{19\text{ hr. }38\text{ min.}}{0\text{ hr. }\ 4\text{ min.}}=295{\cdot}0$

(3) Times spent *active* $=\frac{4\text{ hr. }54\text{ min.}}{0\text{ hr. }58\text{ min.}}=5{\cdot}1$



ig. 1. Activity and the intensity of aggregation of *Tenebrio molitor* in the standard humidity gradient. eft, drier side at 94–97% R.H.; right, moister side at 97–100% R.H. Black areas, time spent inactive; hite areas, time spent in motion, including "virtual inactivity".

ulex fatigans, which reacts strongly to humidity, there is apparently no such fect (Thomson, 1938).

This conspicuous activity difference (ortho-kinesis) cannot, however, be the le factor in the reaction since it will be noted that the absolute times spent by the imals in the *active* state is five times higher on the drier side than on the moister 'able I). This may be accounted for either by a difference in the *rates of ovement* of animals when they are active or by some turning mechanism tending keep the animals on the drier half of the chamber. There is no obvious change of ce of the beetles when they cross from one side of the chamber to the other as in e woodlouse (Gunn, 1937), but in this case a more detailed estimate of the rates of movement can be made from the time marks and their distances apart on th charted tracks. The values for these rates on the two sides of the chamber wer obtained, the distances being estimated with a map-measuring instrument. Ther was found to be no significant difference in the rates of movement of active anima on the drier and moister sides of the chamber. It is therefore clear that the othe possible factor, namely some form of turning mechanism, must be playing considerable part in the reaction. Experiments on this point are described later.

DIFFERENTIAL ACTIVITY IN CONSTANT HUMIDITIES

Differential activity of the type described in the above section may also b exhibited when animals are kept at different constant humidities; at one intensit they are continuously active, at another they remain inactive. This is the case i the woodlouse (Gunn, 1937). The behaviour of *Tenebrio molitor* was examine under these conditions. The apparatus used was the activity chamber, a close vessel kept at a constant uniform humidity. In order to make the results comparable, the vessels used for this purpose were the same as those used in th previous humidity gradient experiments. Five animals were placed in eac chamber and the numbers active and inactive noted after 15 min. The animals wer then activated mechanically by pushing them with a pipe cleaner and the proces repeated twenty times daily so that each experiment yielded 100 records of activity The experiments were carried out at various different humidities and repeate several times at each humidity. The results obtained from data on 300 animals i sixty experiments are shown in Fig. 2. It will be seen that the activity increase sharply as the relative humidity approaches 100%. This is in keeping with th experiments previously described by Pielou & Gunn (1940) on the reactions of th beetles to *differences* of humidity in which the sensitivity increased sharply as th highest relative humidity approached 100%. It will be seen, however, that whe these insects are kept at constant humidities they do not exhibit such a marke difference of activity as is shown in the alternative chamber where there is a gradien of humidity. A further more detailed series of experiments was carried out t examine this point.

The animals were placed in activity chambers as in the above series but th numbers active and inactive were noted at 3, 6, 9, 12 and 15 min. following eac activation. The results are shown in Fig. 3 which are obtained from twenty experiments involving 100 animals and yielding 10,000 activity records. It will b seen that in constant humidity the activity tends to fall to a basal level; the animal are more active in wet air than dry air. But the extremely marked difference o activity seen in the alternative chamber (Fig. 1 and Table I) is much reduced i constant humidities. At the drier side of the alternative chamber the relativ humidity is slightly over 90% and here 80% of the animals' time is spent inactive in a constant relative humidity of 90% approximately 90% of their time is spen inactive (Fig. 3). There is no marked divergence here. But in the alternativ chamber on the wet side (100% R.H.) only 6% of the animals' time is spent inactive a constant relative humidity of 100 % however, 75 % of the time is spent inactive. here appears therefore to be a marked reduction of the activity of animals at 00 % R.H. if they are kept at this humidity even for a short time. This marked duction is no doubt due to sensory adaptation, for in the alternative chamber ey are constantly exposed to changing humidities, for a few centimetres movement y the animals is sufficient to carry them right across the chamber, with no chance adapt.

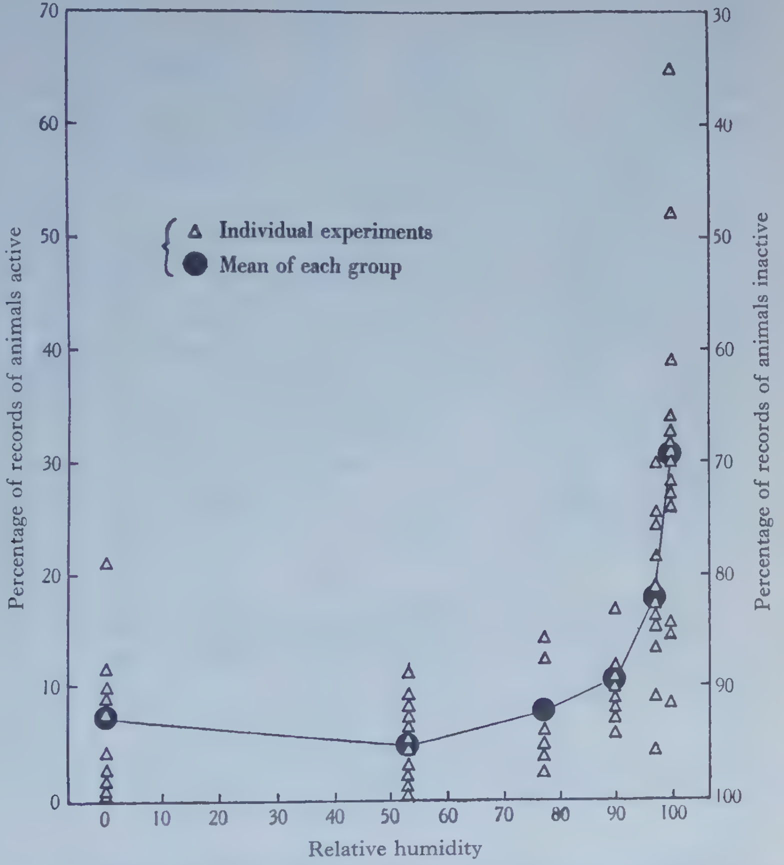


Fig. 2. Activity of *Tenebrio molitor* in uniform humidities. Each triangle represents the proportion of animals active (left) or inactive (right) 15 min. after activation (twenty readings on five animals each), the mean of each group is shown by the large circle.

"VIRTUAL INACTIVITY" IN THE ALTERNATIVE CHAMBER

The track records reveal at once a reaction which intensifies the differential activity mechanism. On the drier side, and only on the drier side, the animals are frequently "virtually inactive" although they are moving about. This is because n this type of movement they turn very frequently and sharply and remain in a small confined area (Fig. 4B); they do not move far, spend much time poking their heads through the holes in the zinc platform and, if they are near the edge of th chamber, trying to climb up the walls. Rather more than one third of the tota time spent in active movement on the drier side is occupied in this type of movemen (Table II). This "virtual inactivity" tends to confine the animals on the drier sid just as does the real inactivity so preponderant here.

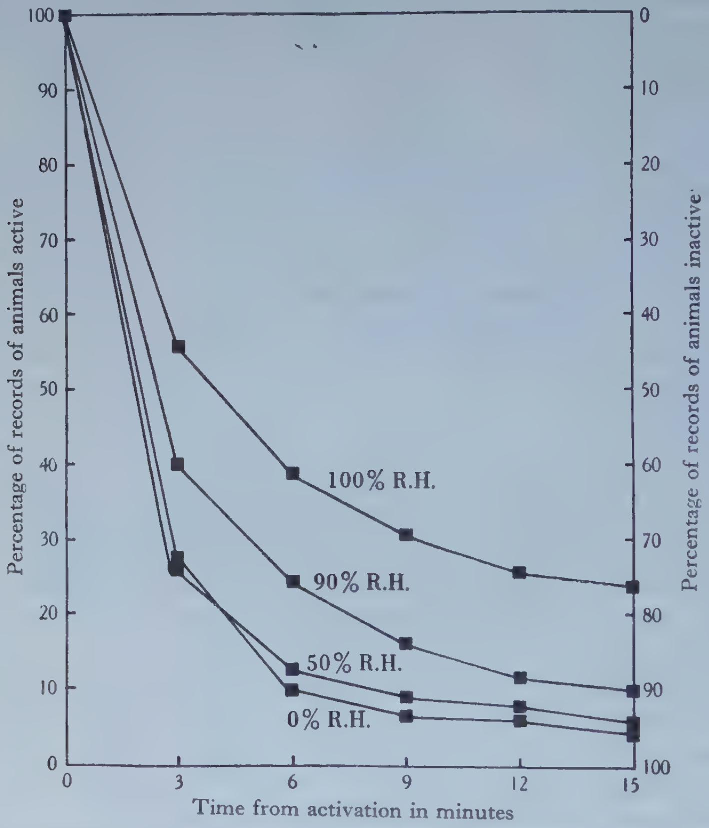


Fig. 3. Decline in activity with time, following activation, in *Tenebrio molitor* kept at various constant and uniform humidities. The activity practically reaches a steady level within 15 min.

ORIENTATION IN THE ALTERNATIVE CHAMBER

It is clear from the data already described that the bulk of the intense reaction is accounted for by a marked differential activity or ortho-kinesis; very frequently the tracks show evidence of no other mechanism (Fig. 4A). The "virtual inactivity" also accounts for part of the reaction (Fig. 4B). Besides these, however, records are also obtained (Fig. 4C, D) which seem to show that some other reaction is at work. These tracks are characterized by frequent turning movements which, in general, tend to keep the animal, when actively walking, on the drier side of the chamber. Such movements must explain why the time spent by the animals in the active state, even after "virtual inactivity" is excluded, is three times greater

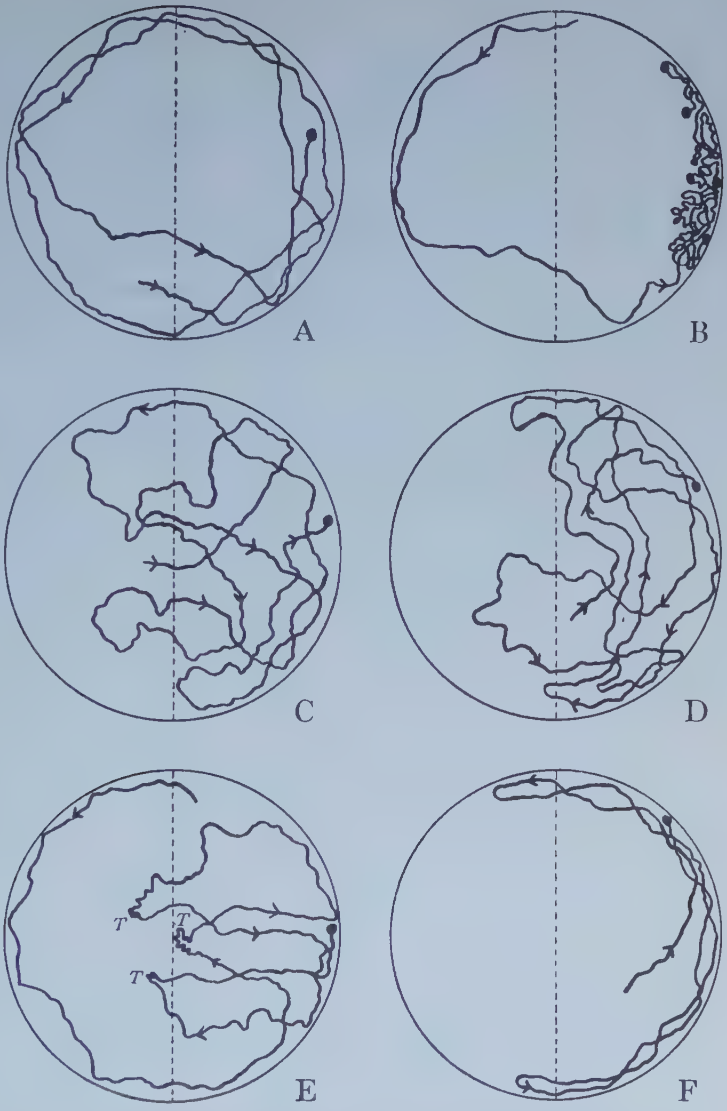


Fig. 4. Examples of paths taken by *Tenebrio molitor* in the standard gradient of humidity. Drier side at 94–97 % R.H. on the right; moister side at 97–100 % R.H. on the left. Black points show where insects came to rest. A, no turning reaction shown, activity mechanism alone bringing animal to rest on drier side. B, "virtual inactivity" on the drier side. C and D, random turning movements (klino-kinesis) bringing animals to drier side. E, reactions with signs of directed component; at *T* beetle hesitated and made trial movements with antennae. F, turning movements shown, although beetle walking in contact with edge of chamber.

on the drier side than on the moister (Table II). These turning movement appear, in general, quite random and undoubtedly they mainly represent a undirected reaction towards humidity, not of the sharply defined and stereotype kind seen in *Paramecium* (Jennings, 1904) but of the more meandering type analyse by Ullyott (1936) in the flatworm *Dendrocoelum lacteum*. These reactions are of th type usually called "phobotaxis" (Kühn, 1919) but are better designated a "*klino-kinesis*" (Gunn *et al.* 1937). In this group of reactions the random movements of the animal as a whole bring it into a successive series of different intensitie of stimulation; there is not necessarily independent movement on the part of th receptors. In a separate series of experiments, the movements of the antenna (on which the humidity receptors are situated (Pielou, 1940)) were observe with a lens and it is clear that this type of bold random movement is accompanie by very little motion of the antennae. This therefore provides additional evidenc for supposing that this type of reaction is undirected.

Table II. *Times spent by* Tenebrio *when active, on the drier and moister sides of the standard humidity gradient alternative chamber. Total times from twenty-two experiments*

	Drier side 94–97% R.H.	Moister side 97–100% R.H.
Total time active	4 hr. 54 min.	0 hr. 58 min.
1. Times spent in normal uninterrupted movement	1 hr. 8 min.	0 hr. 21 min.
2. Times spent in active movement but close to edge of chamber	1 hr. 58 min.	0 hr. 37 min.
3. "Virtual Inactivity"	1 hr. 48 min.	—

Ratios of times spent on the two sides $\frac{\text{Drier}}{\text{Moister}}$

(1) Free uninterrupted movement $= \frac{68}{21} = 3{\cdot}24$

(2) Active movement near edge of chamber $= \frac{118}{37} = 3{\cdot}19$

(3) "Virtual Inactivity" occurred only on the drier side

Apart from these undoubted random and undirected turning movements, ther are some indications of a small directed element in the reaction. Sometime actively moving animals slow up, even stop momentarily, and during this hesitan phase make very varied trial movements with the antennae; this is followed by a fairly precise movement towards the dry side (Fig. 4E; trial movements at *T*) This appears to correspond to the type of behaviour reaction in which the path o the animal is directed with the aid of trial movements of the receptor region of th body into regions of different intensity of stimulation. Here the antennae are th receptor regions and presumably their Johnston's organs provide the animal wit information about the direction in which the antennae are pointing and so can result in "a reflex pursuit of the antennae" (Wigglesworth & Gillett, 1934). This is the type of orientating mechanism now known as *klino-taxis* (Fraenkel & Gunn

940). Such extreme cases as those shown in Fig. 4E are however, rare; only ifteen clear-cut cases were seen in a total of over 25 hr. observation. An example of his type of behaviour is seen in the reaction of *Rhodnius* towards temperature Wigglesworth & Gillett, 1934).

Further evidence on the existence of a slight directed element in the reaction was obtained from a detailed analysis of the directions of the turning movements made by the animals. The tracks particularly examined were those in a central zone 2·5 cm. wide and parallel with the mid-line, where the gradient is steepest. Records were picked out in which the paths of the animals were, for some time, parallel or very nearly parallel to the mid-line; that is, the movements of the animals were approximately at right angles to the direction of the gradient of humidity. If there is no directed element in the reaction any turn from this position should be at random, without bias to left or to right. In many cases there was no turn at all, the animals continuing on, uninterrupted, to the edge of the chamber. Frequently, however, at some point the animal turned to one side or the other. Using 15° as the limit of error in estimating these turns and counting turns to the dry side as positive and those to the moister side as negative, the average angle of turning was +36·0°. This indicates a significant directed reaction towards the drier side; if the directed reaction was maximal the angle would be +90·0°. The average +36·0° was obtained from thirty-four records of such movements in the narrow central zone only, where the turning movements are most intense.

The corresponding data were also abstracted for such turning movements in a similar but wider zone, also central and parallel with the mid-line, 10 cm. in width. From this larger area 124 such records were obtained and here the average resultant angle of turning was +29·0°.

All analysis dealing with turning movements was made from these records in which the animals were walking freely in the chamber. The animals spend a good deal of time walking at a normal rate but close to the edge of the chamber, but the ratio of the times spent on the two sides is the same as when the animals are moving freely in the open space of the chamber (Table II). Moreover, the turning movements are also shown here (Fig. 4F). Presumably the same mechanisms are at work in both cases but the analysis can only be carried out with those track records in which the animals were free from the edge of the chamber.

There is no evidence of a true tropo-tactic element in the reaction; unilateral amputation of an antenna does not result in any tendency to turn to one side (circus movement) either in a gradient of humidity or in constant humidity. There is none of the marked circling of the sort shown by many unilaterally blinded animals when reacting to light, while statistical analysis of the records shows that there was not even a slight tendency to turn to one side. Circus movements in response to odours seem always to be of the latter kind, with a somewhat uncertain meaning, but even these were not found in *Tenebrio*.

SUMMARY

1. The mechanism of the reaction of *Tenebrio molitor* towards humidity ha been analysed into its component behaviour elements. An estimate has been mac of the quantitative importance of each type of behaviour.

2. In a *gradient* of humidity from 94 to 100% R.H. the beetle rarely comes t rest in the moister region but 80% of its time in the drier region is spent motionles

In different *uniform* humidities this differential activity is still evident but ver much reduced.

3. The reaction is intensified somewhat by "virtual inactivity"—restricte movement confined to a small area—which is shown only in the drier part of th gradient.

4. Animals approaching regions of high humidity show turning movemen which have both an undirected component (klino-kinesis) and a directed com ponent involving movements of the antennae (klino-taxis).

5. Tropo-taxis does not occur. There are no circus movements after unilater amputation of the antennae.

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REFERENCES

BARROWS, W. M. (1907). *J. exp. Zool.* **4**, 515–37.
FLÜGGE, C. (1934). *Z. vergl. Physiol.* **20**, 463–500.
FRAENKEL, G. S. & GUNN, D. L. (1940). *The Orientation of Animals: Kineses, Taxes and Compa Reactions*. Oxford.
GUNN, D. L. (1937). *J. exp. Biol.* **14**, 178–86.
GUNN, D. L. & KENNEDY, J. S. (1936). *J. exp. Biol.* **13**, 450–9.
GUNN, D. L., KENNEDY, J. S. & PIELOU, D. P. (1937). *Nature, Lond.*, **140**, 1064.
HARTUNG, E. (1935). *Z. vergl. Physiol.* **22**, 119–44.
JENNINGS, H. S. (1904). *Publ. Carneg. Instn.* no. 16, 256 pp.
KENNEDY, J. S. (1937). *J. exp. Biol.* **14**, 187–97.
KÜHN, A. (1919). *Die Orientierung der Tiere im Raum*, 71 pp. Jena.
—— (1929). Bethe's *Handb. norm. path. Physiol.* **12/1**, 17–35.
MURR, L. (1930). *Z. vergl. Physiol.* **11**, 210–70.
PIELOU, D. P. (1940). *J. exp. Biol.* **17**.
PIELOU, D. P. & GUNN, D. L. (1940). *J. exp Biol.* **17**.
THOMSON, R. C. M. (1938). *Bull. ent. Res.* **29**, 125–40.
ULLYOTT, P. (1936). *J. exp. Biol.* **13**, 253–78.
WARNKE, G. (1931). *Z. vergl. Physiol.* **14**, 121–99.
WIGGLESWORTH, V. B. & GILLETT, J. D. (1934). *J. exp. Biol.* **11**, 120–39, 408.

THE PROTOPLASMIC VISCOSITY OF *PARAMECIUM*

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(With Three Text-figures)

Since Heilbrunn's work in 1926 most workers have agreed that the viscosity of protoplasm is low; all the recent observations, with one exception, have given figures which are not markedly different from each other. The exception, the work of Fetter (1926) on *Paramecium*, gives a viscosity value which is much higher than that found in other material. The present paper constitutes a re-examination of the viscosity values given by Fetter.

MATERIAL AND METHOD

The animals used were obtained from a stock culture of *Paramecium* sp. The experimental method was originally based on that of Fetter, but it required so much modification in detail, that in its final form little trace of the original method remained.

When the animals were fed with particles of different kinds (iron, indian ink, and starch were among those tried), it was found that there was a very great variation in their degree of movement in a centrifugal field. It seemed probable that this was due to differences in the orientation of the animals in the centrifuge tube. Various attempts were made to control this factor, the final method being as follows.

A few cubic centimetres of culture were put into a watch-glass and a small quantity of iron particles mixed with the water. The iron used was the so-called "dust iron" manufactured for use in high-frequency radio circuits. These particles are nearly spherical and between 3 and 4 μ in diameter. They were ingested very readily by the *Paramecium*; after 2–3 min. a majority of the animals had one or more vacuoles containing iron. An animal which had not ingested too much metal was selected under a dissecting microscope and was drawn into a capillary tube about 3–4 cm. long. The bore of the tube was very little greater that the cross-section of the animal; the capillary was sealed at both ends and cemented to a glass slide. The position of the *Paramecium* in the capillary was marked by a line drawn on the slide and the latter placed in a special carrier (Fig. 1). This carrier was rotated by an electric motor at a predetermined speed. At the end of the required time the motor was stopped and the slide examined under a microscope, and the position of a certain vacuole noted. The slide was then replaced in the carrier and the procedure repeated. When the particles had been moved to one end of the animal, the slide was rotated through 180° and replaced in the carrier. The vacuoles could then be moved back towards the end at which they started. This could be repeated many times; actually the largest number of measurements carried out on a single animal was seventeen.

Since the *Paramecium* nearly filled the bore of the tube, their rate of movement along it during centrifuging was slow, so that the radius of rotation, and therefore the centrifugal force, remained constant during an experiment. The amount of movement of the animal could of course be determined by reference to the mark made on the slide before centrifuging. The carrier was graduated in centimetres from the axis, so that the radius of rotation could be read accurately.

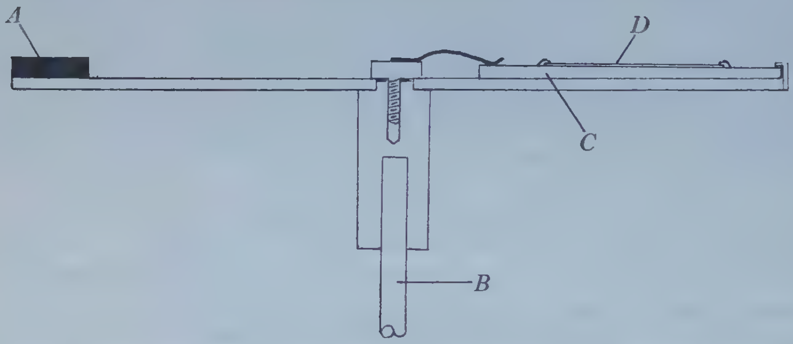

Fig. 1. Centrifuge head to carry slide. *A*, balance weight; *B*, centrifuge shaft; *C*, slide; *D*, capillary tube.

The method of speed control was simple and satisfactory. A black disk with a radial white bar was mounted on the end of the shaft carrying the slide holder, and was illuminated by a neon lamp connected to the 50-cycle A.C. supply. This formed a very satisfactory stroboscope, with which it was possible to maintain a constant speed at various levels from 10 to 50 rev./sec. All the measurable speeds are, of course, submultiples of the hundred flashes a second given by the neon lamp.

The motor speed was adjusted by a resistance before the slide was placed in the carrier; and the current was then switched off without altering the resistance. With the slide in place, the motor was restarted, and the chosen speed reached in a few revolutions.

Within certain limits, the above technique gave results which seemed to be reliable, but irregularities appeared when attempts were made to use very low forces. All attempts to find any constant factor in these irregularities failed; it was decided therefore that a microscope centrifuge would be the only means by which it would be possible to study the effect of low forces.

The choice of instrument lay between the type designed by Harvey (1933), or that described by Beams (1937). These differ greatly in their optical systems, each having its own particular merits; the type due to Beams is carried on the air-turbine centrifuge, while Harvey's instrument has an electric drive. The simplicity and vibrationless running of the air-turbine centrifuge is exceptionally valuable for an optical instrument, but unfortunately the minimum speed obtainable is much too high for the present type of work. On the other hand, the constructional simplicity of Beams's design is a very useful feature. With these and other points in mind, an attempt was made to design a centrifuge which would combine some of the advantages of both types.

The design is shown in Fig. 2. The instrument incorporates the Pickels's (1936) optical system and revolves on a fixed horizontal axis. The advantage of the horizontal mounting lies in the fact that the lighting system can be mounted on the bench where adjustments can be made conveniently; also, the microscope axis can be vertical or inclined at any convenient angle.

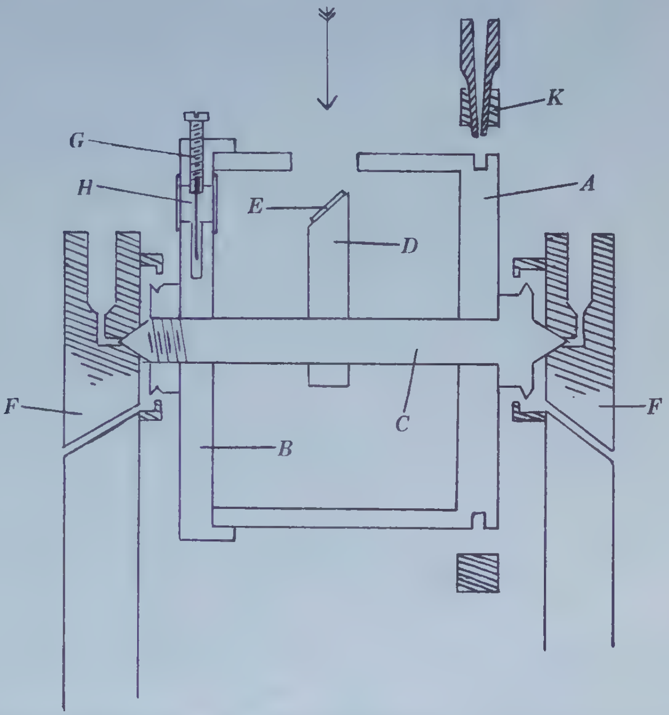

Fig. 2. Direct vision microscope centrifuge (vertical section). *A*, rotor case; *B*, rotor cap containing capillary; *C*, mounting rod; *D*, mirror carrier; *E*, mirror; *F*, supports and bearings; *G*, adjusting screw carrying capillary; *H*, cross-hole closed by cover-slips and filled with clove oil; *K*, ring carrying driving jets. Arrow shows optic axis of microscope.

The rotor is a hollow cylinder (*A*) closed at one end and fitted with a removable cap (*B*) at the other. A steel rod (*C*) runs through the axis and is threaded at one end, a nut on this thread holding the cap in place. The ends of this rod are conical and are case-hardened and polished; these form the bearing surfaces.

The mirror carrier is a brass bar (*D*) fitting tightly on to the rod; this bar is bevelled at its outer end to form a flat surface at 45° to the axis. On this surface a stainless steel mirror (*E*) is fastened.

The stationary frame (F), which carries conical brass bearings, is mounted on the condenser carrier of a microscope and can therefore be centred under the objective. The bearings are fed with oil as shown in Fig. 2; as the oil works through the bearing, it is thrown off the rod and caught in the stationary rings from which it drains into a reservoir.

The method of mounting the specimen is as follows: the cell or cells to be tudied is drawn into a glass capillary as already described. The capillary is then sealed and cemented to the end of a screw (G) that fits into a radial hole in the rotor cap. This hole is crossed by a large hole (H) parallel to the axis, which is closed on each side by a cover-glass. The chamber so formed is filled with cedar-wood oil. The capillary after insertion is adjusted radially to bring the specimen into the centre of the field.

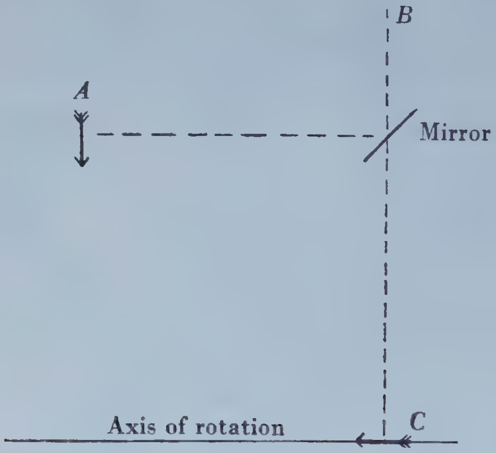

Fig. 3. Optical system. For explanation, see text.

Rotation is effected by compressed air. On the periphery of the rotor is a ring of shallow holes bored at a slight angle to the radius. Attached to the stationary frame is a ring (K) concentric with the rotor; this carries three air jets which are directed at right angles to the axis, and in line with the holes.

The light source is a pointolite lamp, whose electrode is focused on to a slit through a cylindrical lens. The slit is then focused on the object through a low-power microscope objective.

The theory of the optical system is shown in Fig. 3. The object is at A and is observed from B. The virtual image in the mirror is at C. The distances are adjusted so that this image lies on the axis of rotation. The result is, of course, that there is no apparent displacement of the object during the time it is being observed, but only a small rotation which is hardly perceptible.

Since high speeds were not required, the measurement and control of the speed were carried out by means of the disk and neon lamp as described before. The instrument was very simple to use, and, though not entirely satisfactory, it gave a clear solution to the problems for which it was constructed.

OBSERVATIONS

The first point of importance which must be stated, is that the iron particles were found to remain in a vacuole which contained a large proportion of water. This vacuole moved as a whole; consequently the diameter of the moving body is that of the vacuole and not the diameter of the iron particles.

The forces used were much lower than those employed by other workers (50–100 g.), and actually might, with advantage, have been still lower, since the vacuoles moved rather too quickly for satisfactory timing. However, for various reasons, attempts to use lower forces were not successful; very marked irregularities appeared. For example, one application of the force might cause a movement of 20 μ, while the next might produce no effect at all. The observations with the microscope centrifuge are thought to give a possible explanation of this phenomenon.

The calculation of the results was carried out as follows. The viscosity is obtained from Cunningham's (1910) modification of Stokes's formula,

$$V=\frac{2cg(\sigma-\rho)a^2}{9\eta},$$

where c is the acceleration as a multiple of gravity, σ the specific gravity of the particle, ρ the specific gravity of the fluid. a the radius of the particle, and η the viscosity.

The average food vacuole is about 14 μ in diameter and might, for example, contain seven iron particles, 10% of its volume. If we assume that the rest of the vacuole is filled with water, its density as a whole works out at 1·7. Actually there is some food material in the vacuole, but, since the normal vacuole tends to move in a centripetal direction, its density cannot be more than 1·038 that of the animal. This will make no significant difference to the figure already given.

When a *Paramecium* with such a vacuole was subject to a centrifugal force of 67 g., it was found that the average velocity of movement of the vacuole was 0·005 cm./sec. Actually the average distance moved in nine consecutive runs was 160 μ. The variation was about 10%. These figures give a viscosity of 0·5 c.g.s. units. It is considered that this figure is probably too high.

The effect of temperature changes on the viscosity was studied with numerous specimens, but no reliable result could be obtained. It was clear, however, that there was no trace of a maximum around 16° C. as stated by Heilbrunn (1928). The results tended to show a decrease from about 0·9 to 0·1 in passing from 10 to 38°, but were too irregular to give any indication of the rate of change between these temperatures.

The microscope centrifuge gave some useful results. The first point noticed was the effect of using a rather wide tube in which to centrifuge the animals. The *Paramecium* tended to turn obliquely across the tube, so that the vacuole, moving radially, came against the side and moved along it at a very greatly reduced speed. On repeating this with a low force, it was found that, when subjected to about 45 g. or less, the vacuole would cross the animal obliquely and then remain against the pellicle without moving. The movement could be restarted by increasing th force.

It was also possible to see the interference caused to the movement by th nucleus. Further, the movement was sometimes checked for no apparent reason it is suggested that the excretory canals may be responsible for this effect.

With the design of centrifuge used it was not possible to control the speed wit sufficient accuracy to enable any viscosity measurement to be obtained. It i hoped to measure viscosity over small distances by means of a different type c rotor drive which will allow a predetermined speed to be accurately maintaine It seems certain that the average velocity of a body through a large part of th length of the animal is much less than the maximum on account of the numerou obstructions, other food vacuoles, nucleus, and other cell organs which may imped its movement.

DISCUSSION

The centrifuge technique has been attacked by several workers who rely o other methods. Chambers (1924), for example, objects to "The rather drasti action of centrifugal force on the delicately balanced states of viscosity". Actuall the centrifugal force seems to have a surprisingly small effect on the cell. Th most remarkable evidence on this point is given by the work of Guyer & Clau (1936), who studied the redistribution of the components of anterior pituitary cell after ultra-centrifuging at 400,000 g. In this case the cells, which were transplante into the animal after centrifuging, recovered their normal appearance after a fe hours, and were otherwise unaffected.

Since Heilbrunn's forces were only about a hundredth of these, and those use in the present work about a thousandth, it seems safe to assume that the injuriou effects may be neglected.

Heilbrunn (1926) discusses in detail his figures for *Arbacia* eggs. He measure the specific gravity of the granules by finding the sugar solution in which the would just move centrifugally. It seems important to know what variations i density occur between individual granules. Since the relevant term is so small, slight variation in it would make a large difference in the velocity. Also, two sets o eggs gave values for the protoplasmic density, differing by about 30%. It seem strange that this difference was not considered to be significant, the measurement being averaged to calculate the viscosity. When eggs of the same species differ i density by 30% it is not unreasonable to expect some other measurable physica or chemical difference.

Heilbrunn, in this work, also makes an estimation of the Cunninghan correction for the interference between the granules. He apparently does n take into account the fact that, as the movement proceeds, the granules becom closer together and their mutual interference greater. Thus the correction migh be more accurately calculated from an average spacing and not, as is done, from th maximum spacing of the granules.

On the whole the experiments show clearly that the viscosity of these marin eggs is very low, and that it is not therefore comparable to that of glycerine as stated by Seifriz. It is therefore an obvious inference that this author's method of inserting comparatively large nickel spheres into the eggs, has some very marked effect on the physical state of the protoplasm.

It is suggested that these methods, which are dependent on the introduction of bodies into the cell, are only valid in the particular case where the cell is accustomed to ingest solid particles during its normal life, as in some Protozoa and Myxomycetes. The low value obtained for the protoplasm of the latter is evidence that the organism was not seriously affected by the introduction of inert bodies. The same principle is applicable in the present case; *Paramecium* normally ingest solid bodies, so that we need not expect any disturbance to arise from Fetter's method of making them ingest heavy particles.

As previously mentioned, Fetter's results are not comparable to any others obtained by centrifuge technique. It would appear then that Fetter's work needs careful consideration.

Unfortunately her wording is not at all clear on several important points. First, there is no mention of the fact that the iron is in a vacuole. Fetter states that "The density of the iron moistened as it would be in the body was found to be 1·5". Apparently the iron was an amorphous powder, and its density when saturated with water was found. The density of the vacuole as a whole would then be much less. Even assuming that the vacuole was three-quarters full of the water-saturated iron, its density would be 1·12 instead of 1·5. This would make a difference of nearly 600 % in the result. It seems certain that Fetter did not notice the vacuole, since, when using starch, the density used in the calculation is that of the starch; if a vacuole had been allowed for, the amount of water would have been taken into account.

As well as this difficulty the technique itself introduces a serious error. As has been shown, the vacuole is greatly retarded as it approaches the pellicle. It is unlikely that more than a very small proportion of the animals in a wide tube would become fixed in a position with their long axis parallel to the centrifugal force, yet only those few could give even an approximate indication of the true velocity of movement. It is apparent, therefore, that the results may have little or no meaning.

While it is considered that the present work gives a more reliable result, it appears certain that the value obtained is a maximum for the following reasons. The density and diameter of the vacuoles can be determined with some accuracy. In the particular case mentioned the density cannot be less than 1·7 or more than 1·73. The diameter (14 μ) is sufficiently large to be measured without serious error. The applied force can also be known within narrow limits. On the other hand, the technique does not give a reliable value for the velocity of movement, since it does not take into account the retardation caused by various intracellular structures. It does, however, give a minimum value for this speed, and therefore a maximum for the viscosity.

It is possible that the complex structure of a protozoan cell may invalidate the use of a moving body as large as the vacuole. Fetter suggested that the hig viscosity which she found might be due to the presence of a neuromotor system a described by Rees (1922). On the other hand, Lund (1933) has stated that th neuromotor system in *Paramecium* is located almost entirely at the periphery If this is so, the structure could not have any effect on the viscosity measurement

Nevertheless, there is a little understood state of organization in the protozoa cell (with great variation between different species) which, unless the movement i studied in different parts of the cell, might cause large and incalculable errors in th viscosity measurement.

One point, slightly outside the scope of this work, on which the microscop centrifuge has given some evidence, is the condition of the material underlying the pellicle. The fact that the vacuole, moving under the action of a low force, stop when it reaches this layer, and requires a higher force to restart it, is evidence tha the substance in this region is of an elastic or plastic nature.

SUMMARY

1. An improved technique for measuring the protoplasmic viscosity of certai types of cell is described.

2. It is shown that the viscosity of *Paramecium* is not greater than 0·5 c.g.s unit, and that it may be much less.

3. A simplified type of microscope centrifuge has revealed several sources o error in the centrifuge method customarily employed for the determination o protoplasmic viscosity.

REFERENCES

Beams, H. W. (1937). *J. Mar. Biol. Ass. U.K.* **21**, 571–88.
Chambers, R. (1924). *Amer. J. Physiol.* **43**, 1–12.
Cunningham, E. (1910). *Proc. roy. Soc.* A, **83**, 357.
Fetter, D. (1926). *J. exp. Zool.* **44**, 279–83.
Guyer, M. F. & Claus, P. E. (1936). *Biol. Bull. Wood's Hole*, **71**, 462–8.
Harvey, E. N. (1932). *Science*, **75**, 267.
—— (1933). *Science*, **77**, 430.
Heilbronn, A. L. (1922). *Jb. wiss. Bot.* **61**, 284–338.
Heilbrunn, L. V. (1921). *J. exp. Zool.* 417–47.
—— (1926). *J. exp. Zool.* **43**, 313–20.
—— (1926). *J. exp. Zool.* **44**, 255–78.
—— (1928). *Protoplasma Monographien*, **1**. Berlin: Borntraegar.
—— (1930). *Protoplasma*, **8**, 596–618.
Lund, E. E. (1933). *Univ. Calif. Publ. Zool.* **39**, 35.
Pickels, E. G. (1936). *Science*, **83**, 471.
Rees, C. W. (1922). *Univ. Calif. Publ. Zool.* **20**, 333–64.

THE TOXICITY OF THE DOUBLE CHLORIDES OF MERCURY AND SODIUM

. EXPERIMENTS WITH THE MINNOW *PHOXINUS PHOXINUS* (L.)

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(With Two Text-figures)

INTRODUCTION

MERCURIC chloride is generally recognized as one of the most toxic of inorganic substances, and one of the most powerful of antiseptics. Thus in a study by Martindale & Westcott (1921, pp. 348–9) of the antiseptic value of thirty-two substances, the germicidal power of mercuric chloride was found to be equalled only by mercuric potassium iodide. *Bacillus coli* was the organism employed.

With sodium chloride mercuric chloride forms two double salts, $NaHgCl_3$ and Na_2HgCl_4. Little study appears to have been made of the toxic power of complex salts, but the general conclusion to be drawn from the evidence available is that complex salts are less toxic than simple salts containing the same elements. Thus it s generally recognized that ferricyanides and ferrocyanides are less toxic than cyanides. Heald (1896) found potassium ferrocyanide fifty times less toxic to *Pisum* seedlings than potassium cyanide; Kahlenberg & True (1896) have shown that the complex ion $AgC_2N_2^-$, formed by the dissociation of potassium argenticyanide $KAgC_2N_2$, is considerably less toxic than either the silver ion Ag^+ or the cyanide ion CN^-, and that the complex copper ion of Fehling's solution is over a hundred times less toxic to seedlings of *Lupinus albus* than the copper ion of copper sulphate. The toxicity of the double chlorides of mercury and sodium is of particular nterest for whereas mercuric chloride is a non-electrolyte, and presumably does not dissociate into ions, the double chlorides ionize in aqueous solution in the following way:

$$NaHgCl_3 \rightarrow Na^+ + HgCl_3^-,$$
$$Na_2HgCl_4 \rightarrow 2Na^+ + HgCl_4^=,$$

he sodium thus forming the cation and the mercury and chlorine complex anions. t has been claimed that the ionization of mercuric chloride effected by the addition of sodium chloride results in a considerable increase of toxic activity; thus Clark 1901) states that the toxicity of mercuric chloride to fungus spores can be increased fivefold on the addition of NaCl. On the contrary, Martindale & Westcott (1921, pp. 356–7) conclude that the formation of the double salts in question has n particular effect on the antiseptic power of sublimate in the case of *Bacillus co* and effects a reduction of antiseptic power in the case of anthrax spores. Rona Michaelis (1919) showed that the adsorption of mercuric chloride is greatly d pressed in the presence of sodium chloride because the complex ions $HgCl_3^-$ ar $HgCl_4^=$ are much less readily absorbed than $HgCl_2$ molecules, and Freundlic (1926, p. 212) remarks that the toxicity of sublimate is therefore lowered on th addition of NaCl. Unfortunately, Freundlich does not specify any particul organism.

The controversy regarding the toxic activity of these double chlorides ha prompted the present study, in which the minnow is used as a test animal.

THE TOXICITY OF MIXED SOLUTIONS OF $HgCl_2$ AND NaCl

In all the following experiments small minnows 18–20 mm. in length we used, and were found to give very consistent results provided that they were n kept in the laboratory for more than 5 days before use. The solutions, stock ar experimental, were prepared with water distilled in glass; the salts were "AnalaR" quality and the mercuric chloride was recrystallized before use. Th fish were washed in two changes of glass-distilled water before being transferred t the solutions.

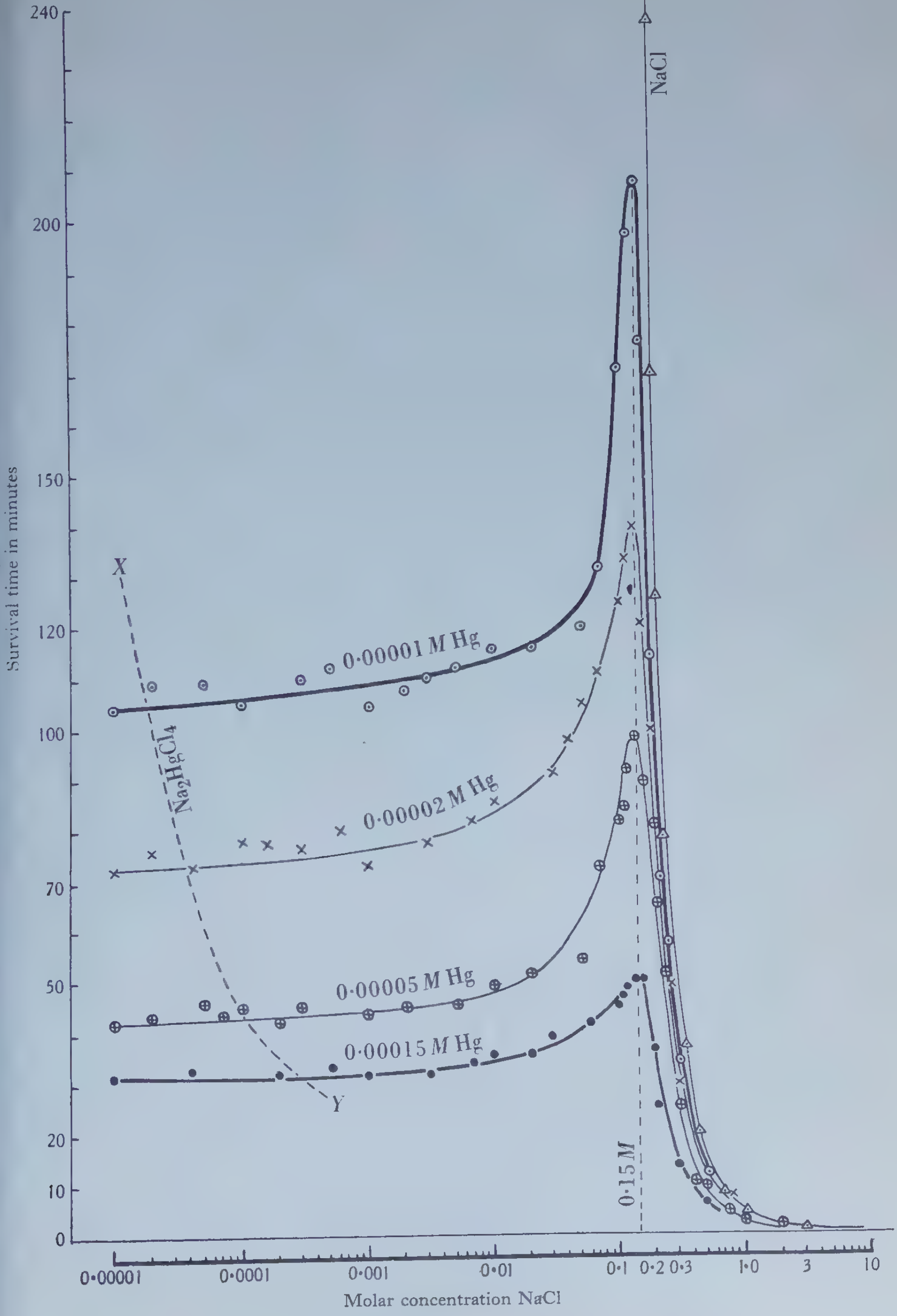
The survival curves for mercuric chloride and for sodium chloride are normal type, the survival time increasing steadily on dilution. The curve fo $HgCl_2$ rises from 15 min. at $10^{-3} M$ to 230 min. at $5 \times 10^{-6} M$. That for NaCl drawn in Fig. 1.

In Fig. 1 four survival curves for mixtures of mercuric chloride and sodiu chloride are drawn. In each series of experiments the molar concentration $HgCl_2$ is maintained at a selected value while that of NaCl is progressively increase from nil to 0·5 – 2·0 *M*. The NaCl concentration scale is logarithmic; since zer cannot be represented on a logarithmic scale NaCl nil is represented as $10^{-5} M$.

Along the line *XY* the composition of the solution corresponds to the formu Na_2HgCl_4. It will be seen that the addition of sufficient NaCl to convert the who of the $HgCl_2$ into the complex salt has no appreciable effect on the toxicity of th solution, and that the only effect of adding ten times as much NaCl is a slig prolongation of the survival time. On the addition of a great excess of Na marked antagonism becomes evident, the survival time rising considerably, ar each curve attains a maximum at approximately 0·15 *M* NaCl. Thereafter th curves descend rapidly, following closely the survival curve for NaCl. At concentr tions of $HgCl_2$ greater than 0·00015 *M* the addition of small quantities of Na remains ineffective, while the antagonism produced on the addition of an exce becomes less marked; at $HgCl_2$ concentrations below $10^{-5} M$ the antagonist effect of an excess of NaCl becomes increasingly pronounced.

At $10^{-5} M$ the solution volume employed contains only 0·02 mg. Hg per fis

Fig. 1. Survival curves for 10^{-5}, 2×10^{-5}, 5×10^{-5} and 15×10^{-5} molar mercuric chloride plus increasing molar concentrations of sodium chloride. NaCl, survival curve for sodium chloride. Each plotted point represents the mean survival time of three minnows in 30 c.c. of solution. Temp. 16°C.



This quantity appears to be very small, but it is found that increasing the solutio volume to 500 c.c. per fish results in no appreciable shortening of the surviv times.

THE RELATION BETWEEN THE TOXICITY OF THE SOLUTIONS AND THEIR OSMOTIC PRESSURE

At 0·15 *M*, the concentration of sodium chloride at which the maximu antagonistic effect is evident, the osmotic pressure of the solution is approximate 6·3 atm. This solution is probably very nearly isotonic, for Ellis (1937, p. 40 states that the osmotic pressure of the blood of fresh-water teleosts is generall 6 atm. The results suggest that the prolongation of the survival time is connecte in some way, with the osmotic pressure of the solution, and this impression materially strengthened by the fact that if increasing quantities of glucose, instea of sodium chloride, are added to mercuric chloride, survival curves of the sam type are obtained. Two survival curves for $HgCl_2$ plus glucose are drawn in Fig. the survival curve for $5 \times 10^{-5} M$ $HgCl_2$ plus NaCl is repeated in this figure f comparison. The upper curves indicate the osmotic pressure of the solutions; th values are calculated from the concentrations (and in the case of NaCl from values), and are necessarily somewhat approximate.

It will be noted that in the $HgCl_2$ plus NaCl and $HgCl_2$ plus glucose curves th maximum survival time is attained at much the same osmotic pressure, (6·3 atm NaCl, 6·7 atm. glucose). A greater molar concentration of glucose than of NaCl necessary to effect the same rise of osmotic pressure because of the ionization of th salt. If the survival curves are plotted with osmotic pressures as abscissae, cor responding curves for $HgCl_2$ plus NaCl and $HgCl_2$ plus glucose are almost coinciden

In the strongly hypertonic solutions, whatever their composition, the death c the fish appears to result from rapid withdrawal of water from the gill tissue with the consequent arrest of the branchial circulation (Jones, 1939, pp. 431–2 At death the gills are clear of mucus film, are bright red and visibly shrunken; th body surface acquires an opalescent appearance which is particularly evident in th cornea and crystalline lens of the eye. In hypotonic, isotonic and slightly hyperton solutions of $HgCl_2$, $HgCl_2$ plus glucose and $HgCl_2$ plus NaCl the death of the fish i accompanied by precipitation of the gill secretions, with consequent asphyxiatio but when the osmotic pressure of the solution is 2–8 atm. the formation of th asphyxiating film of coagulated mucus appears to be delayed. The reason for this i not clear; chemical action between mercuric chloride and glucose does not appea to be a feasible explanation and experiment has shown that the toxicity of othe heavy metal salts is similarly lowered when the solution is rendered isotonic by th addition of glucose or sodium chloride. The osmotic pressure which exists betwee the blood of the freshwater teleost and its normal medium results in a continua influx of water through the gill membranes, the excess of water being eliminated i the dilute and abundant urine (Smith, 1930). If the medium is rendered isoton this absorption of water should cease and it is possible that under these condition gill secretions coagulated by a heavy metal salt may be more readily disperse

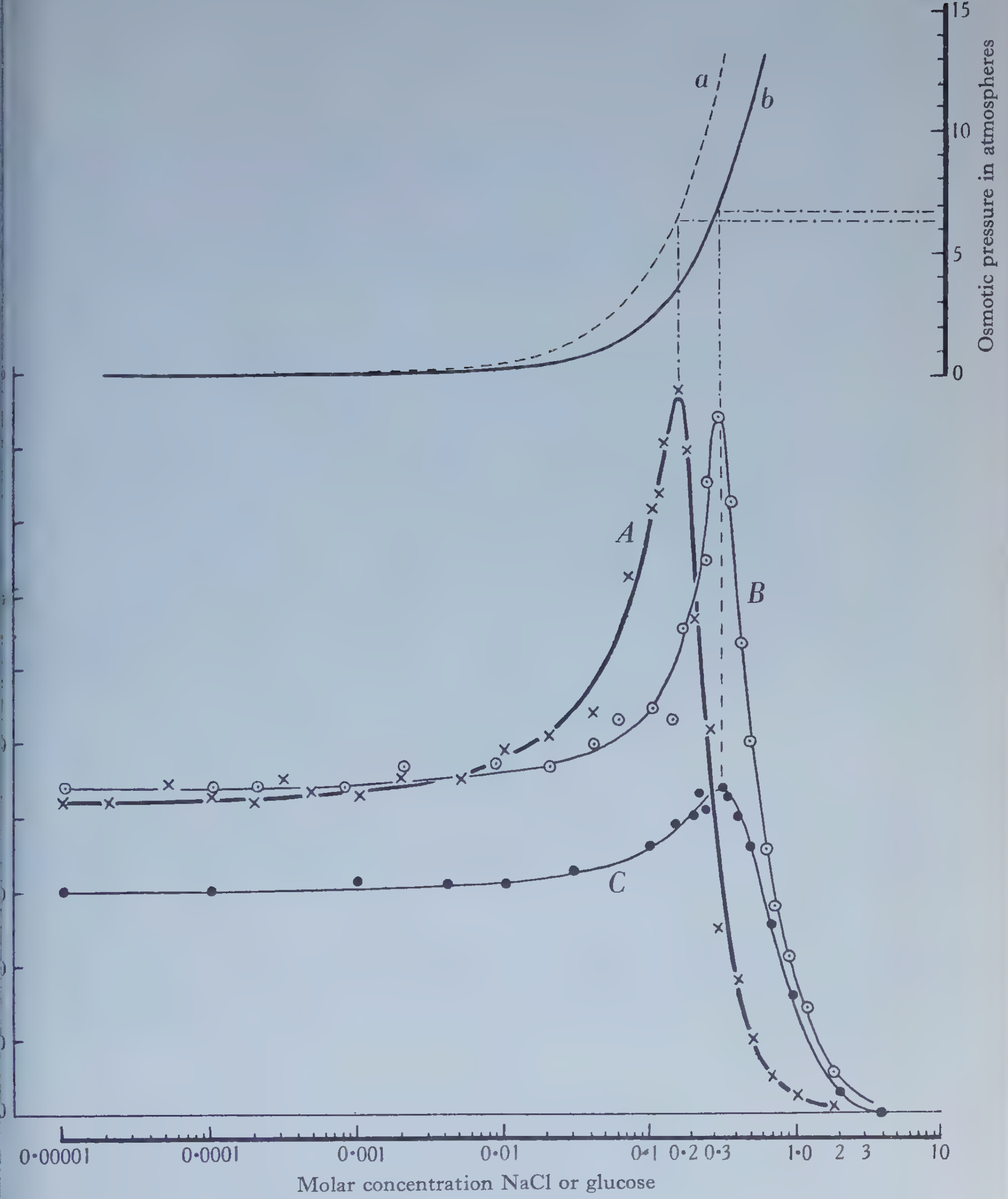


Fig. 2. *A*, survival curve for 5×10^{-5} *M* $HgCl_2$ plus increasing molar concentrations of NaCl; *B* and *C*, survival curves for 5×10^{-5} and 2×10^{-4} *M* $HgCl_2$ plus increasing molar concentrations of glucose. *a*, osmotic pressure of $HgCl_2$ plus NaCl solutions; *b*, osmotic pressure of $HgCl_2$ plus glucose solutions. Other details as Fig. 1.

The problem thus requires further investigation, but the results are interesting that they present a case in which the antagonistic action of a salt appears to resu from the physical, rather than the chemical, changes its addition brings about the nature of the toxic solution.

SUMMARY

1. Mercuric chloride, one of the most toxic of inorganic substances, does no ionize in aqueous solution, but in the presence of sodium chloride forms the doub chlorides $NaHgCl_3$ and Na_2HgCl_4 which ionize readily. It has been various claimed that the ionization of mercuric chloride, thus effected, is accompanied b an increase of toxicity, by a decrease of toxicity, or that the toxicity of the solutio remains unaltered.

2. In an investigation of the toxicity of mixed solutions of $HgCl_2$ and NaCl t the minnow, *Phoxinus phoxinus* (L.), it is found that the addition, to $HgCl_2$ solution of sufficient NaCl to convert the whole of the $HgCl_2$ into the double chlorid Na_2HgCl_4, and even ten times this quantity of NaCl effects no marked change i the toxicity of the solution.

3. On the addition of a considerable excess of NaCl a marked prolongation o the survival time occurs, the maximum antagonistic effect being evident when th solution is approximately isotonic. Almost exactly the same result is obtained if th NaCl is replaced by quantities of glucose sufficient to effect equal changes in th osmotic pressure of the solutions, and thus the antagonistic action of the sodiu chloride appears to be due to the physical, rather than the chemical, changes i addition brings about in the nature of the toxic solution.

REFERENCES

CLARK, J. F. (1901). *J. phys. Chem.* **5**, 289.
ELLIS, M. M. (1937). *Bull. U.S. Bur. Fish.* **48**, no. 22.
FREUNDLICH, H. (1926). *Colloid and Capillary Chemistry*, English translation of 3rd German ed. b H. S. Hatfield. London.
HEALD, F. H. (1896). *Bot. Gaz.* **22**, 125.
JONES, J. R. E. (1939). *J. exp. Biol.* **16**, 425.
KAHLENBERG, L. & TRUE, R. H. (1896). *Bot. Gaz.* **22**, 81.
MARTINDALE, W. H. & WESTCOTT, W. W. (1921). *The Extra Pharmacopoeia*, **2**, 17th ed. London.
RONA, P. & MICHAELIS, L. (1919). *Biochem. Z.* **97**, 85.
SMITH, H. W. (1930). *Amer. J. Physiol.* **93**, 480.

THE EFFECTS ON THE FAT AND STARCH METABOLISM OF *GEBIA* BY THE PARASITE *GYGE BRANCHIALIS*

By T. E. HUGHES, M.A.

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INTRODUCTORY

AN account of the effects of *Gyge branchialis* on its Decapod host has been published by Tucker (1930). He points out that as in the case of *Inachus* parasitized by *Sacculina*, there is a feminization of infected males but no corresponding masculinization of infected females. Unlike *Inachus*, *Gebia* continue to moult after infection, which occurs in the majority of cases when the carapace is between 3 mm. and mm. long. The infected animals attain normal adult size. In this respect they would appear to occupy a position intermediate between *Inachus* and *Eupagurus* parasitized by *Peltogaster* (investigated by Potts, 1906); the infected *Eupagurus* moult apparently rather more frequently than the uninfected.

The effects of parasitization on the general metabolism of the host have been investigated in the case of *Inachus* and other brachyuran crabs by Smith (1911, 913) and Robson (1911). They found that sexually mature female crabs differ from males in having an excess of fatty material in the liver, which in the case of *Inachus* also floods the blood. In parasitized specimens of *Carcinus* (Smith) and *Inachus* (Robson) an excessive production of fat in the liver was found to occur.

Since the effects of the simple blood-sucking *Gyge* upon the morphology of its host are at least as marked as the influence of *Sacculina* on *Inachus*, the changes in the metabolic processes should be equally well marked. The following piece of work was undertaken in order to try to discover whether such changes did in fact occur.

MATERIAL

The specimens used were obtained from Naples, those for a study of the fats and fatty substances being preserved in formol in sea water, the specimens investigated for starch in 70% alcohol.

It may be objected that preserved material might well give unreliable results, but the fixation might be reasonably expected to affect parasitized and unparasitized animals alike, having no very marked effect on their relative fat or starch contents.

METHOD

The method of grouping the animals used for a quantitative estimation of t total fat content was this:

(1) Normal males.
(2) Parasitized males.
(3) Normal females.
(4) Parasitized females.

The normal females were then subdivided into two groups:

(*a*) Those carrying ova attached to the abdominal appendages or with ovar showing orange with well-developed ova through the body wall.

(*b*) Those showing no visible sign of ova.

METHOD OF FAT ESTIMATION

This was done gravimetrically, the fats and allied substances being estimat *en masse* as an ether soluble extract.

A known weight of *Gebia* was saponified by boiling with excess of strong KO solution under a reflux condenser. The fatty acids were then liberated by the acti of concentrated H_2SO_4 on the solution of soaps when it had cooled. An extra was then made by shaking the mixture with a known volume of petroleum ether f 2 hr. On standing, the solution of fatty substances in ether formed an orang yellow layer on top of the contents of the flask. This layer was raised into the ne of the flask by the addition of distilled water and a known volume of the eth solution was pipetted off into a dry weighed beaker. The ether having been evaporat off over a water-bath, the beaker and residual fat were weighed. From this t quantity of fat in the sample of *Gebia* was calculated. About fifteen *Gebia* we taken in each sample, weighing 10–20 g.

METHOD OF GLYCOGEN ESTIMATION

The glycogen was estimated as glucose volumetrically by titration agai Benedict's solution. A known weight of *Gebia* was boiled, with strong KO solution to break up the tissues, for 4 hr. under a reflux. The glycogen was th precipitated by alcohol and separated by filtration. After washing with alcohol a ether the precipitate was dissolved in boiling water and hydrolysed to glucose refluxing with HCl for 2 hr. The solution of sugar thus obtained was then made to a known volume and titrated against Benedict's solution. From the amount sugar in the sample the quantity of starch was then calculated.

RESULTS

Gebia. *Fat expressed as a percentage of body weight*

	Normal male	Parasitized male	Ovigerous female	Non-ovigerous female	Parasitized female
	0·9	1·55	1·80	1·193	1·065
	1·02	1·66	1·795	1·217	1·075
	1·00	1·42	2·510	1·184	1·087
	0·93	1·49	2·210	1·223	1·023
	1·21	1·74	2·733		1·610
	1·16	1·81	2·755		1·380
	0·97		1·930		1·190
	1·06		1·930		1·360
	1·09				
Mean	1·04	1·61	2·21	1·204	1·22
Variance δ^2/N	0·0094	0·0188	0·1485	0·000262	0·0371
S.D. $\pm\sqrt{V}=\sigma$	0·09694	0·1371	0·3854	0·016	0·1926
S.E. $\pm\sigma/N$	0·03231	0·05599	0·1362	0·004	0·06813

Percentage of fat found in 130 specimens of *Gyge* which weighed 7·2 g. was 4·4 %.

Gebia. *Starch expressed as a percentage of body weight*

	Normal female	Parasitized female	Normal male	Parasitized male
	0·1831	0·1819	0·1148	0·1418
	0·1802	0·1921	0·1148	0·1658
	0·1816	0·2260	0·1389	0·1540
	0·1898	0·2165	0·1412	
	0·1889			
	0·1879			
	0·1906			
	0·1890			
Mean	0·1864	0·2041	0·1276	0·1538
Variance δ^2/N	0·000014	0·000317	0·000164	0·000096
S.D. $\pm\sqrt{V}=\sigma$	0·003741	0·01799	0·01266	0·009797
S.E. $\pm\sigma/N$	0·000467	0·00449	0·00316	0·003265

STATISTICAL ANALYSIS OF RESULTS

Fats

Comparison of the normal and parasitized ♂:

$t=8{\cdot}45$.
$n=13$.
P is less than 0·01, $\therefore$ the difference in the means is significant.

Comparison of the non-ovigerous ♀ and normal ♂:

$t=11\cdot93$.

$n=13$.

P is less than 0·01, ∴ the difference in the means is significant.

Comparison of the non-ovigerous and parasitized ♀:

$t=0\cdot1894$.

$n=10$.

P is greater than 0·8, ∴ the difference in the means is not significant.

Starches

Comparison of normal ♀ and parasitized ♀:

$t=7\cdot240$.

$n=10$.

P is less than 0·01, ∴ the difference in the means is significant.

Comparison of normal ♂ and normal ♀:

$t=10\cdot91$.

$n=10$.

P is less than 0·01, ∴ the difference in means is significant.

Comparison of normal ♂ and parasitized ♂:

$t=6\cdot067$.

$n=5$.

P is less than 0·01, ∴ the difference in means is significant.

DISCUSSION OF THE RESULTS

Tucker has reviewed the literature on parasitic castration up to 1930 and ha reached the conclusion that Smith's theory of metabolic stimuli, notwithstandin the criticisms that have been made of it, is still the only one which affords a satisfactory explanation of the facts. The present results appear to be in entire agreemen with and to afford additional support to Smith's theory.

Considering first the fat analyses, it is evident that there is a significant differenc in fat content between normal male and non-ovigerous females. There is a definit increase in fat in parasitized males associated also with the presence of typicall feminine secondary sexual features. There is no increase in fat in parasitized female ovigerous females have a fat content considerably in excess of non-ovigerous one Parasitism of the female is associated with a degeneration and disappearance of th ovary but no change in secondary sexual features. The gonad in infected mal shows an increased number of the oocytes often found in normal testes and th testicular tissue may degenerate and disappear (Tucker, 1930).

The effect of the parasitic *Gyge* on the male *Gebia* seems to consist of creatin an insistent demand for fats which it withdraws from the blood of its host, thereb stimulating the anabolic processes and causing a rise in fat content from 1·04 t

·6%. That *Gyge* does remove fat from its host is shown by the high percentage 4·4) which it contains, and which must originally have come from the host.

In the case of the parasitized females, the fat production of the host appears to e sufficient for host and parasite, but insufficient for host, parasite and ovary. The natural result of this is degeneration of the ovary due to malnutrition. The arasite in the Cryptoniscid stage fixes on to a *Gebia* of a carapace length of less han 6·5 mm., consequently it seems legitimate to assume that the growing *Gyge* rings a metabolic drain to bear on the host of the same nature and at least the ame magnitude as a functional ovary. The growing female parasite is itself deeloping relatively large ovarian structures and it seems feasible to assume that it eeds similar substances in this respect to its host.

The parasitized females do not ever show the great increase in fat content found n ovigerous females; this may be due to one of two causes:

(1) There is no accumulation of fat in the body as in the case of an ovary rammed with yolk-filled eggs.

(2) Whatever stimulus a mature ovary, developing eggs, has on fat production s effectively removed.

Of these two alternatives, I consider the former the more probable. We know hat in the parasitized male there is considerable stimulus to fat production and t seems likely that this is the general effect of the parasite, but that in the female he anabolic processes are unable to supply fat for host, parasite and ovary, the atter degenerates and consequently there is no ovarian accumulation of fats as n an ovigerous female. That *Gyge* creates a demand for fat at least equal to a resting vary is shown by the fact that parasitized females contain as much fat as non-vigerous females, i.e. females with ovaries in an inactive state.

There is a certain amount of evidence to show that the female formative subtance (hormone of Goldschmidt) is involved in some way with fat production. Vhether in parasitized males or in unparasitized females there is an accentuation f feminine features and there is an increase in fat content which is due presumably o an increase in hormone production. The maturation of the ovary causes an ncrease in fat production, and at the same time the setae appear on the first bdominal appendage (Tucker, 1930). In parasitized males, fat production is timulated and at the same time feminine characters make their appearance. The vidence supports the view that the production of female hormone or sexual ormative substance is involved with the production of fatty substances and that hanges brought about in this aspect of anabolism are responsible for the sexual nversions found in parasitized males. It is, of course, possible to regard the fact s a secondary factor and to relate the hormonic changes to some more subtle and itherto undiscovered change in metabolism. At the time of writing there is no irect evidence in support of this view. On the evidence Smith's assumption was n the main correct. The parasite by bringing a demand of a similar nature to that f an ovary to bear on the animal produces such changes in metabolism as lead to feminization of infected males, or an accentuation of feminine features in infected emales where such metabolic changes occur.

As regards the starch content of *Upogebia*, a definite difference exists betwee normal males and females. Parasitization is followed by a slight increase in bo sexes. Smith correlated the inability of *Inachus* and *Carcinus* parasitized *Sacculina* to moult, with the inability to store glycogen owing to demands on i metabolism, by the parasite. This view is further supported by the fact that i ability to moult and approach of maturity of the parasite coincide. *Gebia* goes o moulting after infection and its glycogen content is maintained as high, if n higher, than in uninfected animals. These facts are in support of Smith's views b a further analysis of animals at various stages of ecdysis would be needed to establi a connexion between glycogen anabolism and chitin production. There is eviden that in Insecta glycogen and chitin production are linked (Paillot, 1938). T slight increase of glycogen in parasitized animals makes it conceivable that moulti might occur more frequently in these than in normal animals. There is no eviden that this is so. Potts (1906) was of opinion that *Eupagurus* parasitized by *Peltogast* moulted more frequently than normal. The effect of *Peltogaster* on the metabolis of the host has not yet been investigated, but the conditions in *Gebia* suggest th increased frequency of moulting is not *a priori* impossible. It is, of course, possib that the increase in glycogen is merely due to regression of the gonad lessenin glycogen katabolism, rather than an active stimulus to anabolism of glycogen b the parasite's presence.

Owing to the methods employed larger numbers of animals are needed f glycogen estimation than I had at my disposal, and I therefore hesitate to give an definite opinion on the relative increase of glycogen due to parasitization. I thin it fairly certain that a slight increase does occur.

SUMMARY

1. Males of *Upogebia littoralis* parasitized by *Gyge branchialis* show a mark increase in fat content from 1·04 to 1·6%.

2. Parasitized females have a fat content practically the same as that of ordina non-ovigerous females, 1·2%.

3. Ovigerous females have a fat content considerably higher than that parasitized males or non-ovigerous females, namely 2·2%.

4. The parasite *Gyge* has a relatively high fat content, 4·4%.

5. There is a significant difference in glycogen content between normal mal and females.

6. There may be a slight increase in glycogen following parasitization.

7. The results are essentially in agreement with the theory of metabolic stimu put forward by Geoffrey Smith.

REFERENCES

Goldschmidt, R. (1923). *Physiology of Sex Determination.*
Paillot, A. (1938). *C.R. Soc. Biol., Paris*, **127**, 1502.
Potts, F. A. (1906). *Quart. J. micr. Sci.* **50**, 599.
Robson, G. C. (1911). *Quart J. micr. Sci.* **57**, 267.
Smith, G. (1911). *Quart. J. micr. Sci.* **57**, 251.
—— (1913). *Quart. J. micr. Sci.* **59**, 267.
Tucker, B. W. (1930). *Quart. J. micr. Sci.* **74**, 1.

PHYSIOLOGICAL EFFECTS OF A HYPOTONIC ENVIRONMENT

I. THE ACTION OF HYPOTONIC SALINES ON ISOLATED RHYTHMIC PREPARATIONS FROM POLYCHAETE WORMS (*ARENICOLA MARINA, NEREIS DIVERSICOLOR, PERINEREIS CULTRIFERA*)

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(With Eight Text-figures)

UMEROUS studies have been published on the extent to which aquatic animals can aintain internal environments differing in osmotic pressure from their surroundngs. In the case of polychaete worms, it has been shown that some species, specially *Nereis diversicolor*, can maintain a considerable degree of hypertonicity, ven in very dilute brackish water, while in others, such as *Arenicola marina*, the ternal and external fluids are always isotonic (Schlieper, 1929; Beadle, 1937; enkewitch, 1938). These facts have been correlated with the extent to which the arious species can invade brackish water. In one respect, however, our knowedge is at present very deficient. Remarkably little—in the case of polychaete orms, nothing—is known about the extent to which the tissues of animals can olerate dilution of their bathing media. Information on this question is evidently ecessary if the significance of the body-fluid data is to be properly understood.

The writers were studying the action of ions on an isolated rhythmic preparation om *A. marina*, and, for the reasons stated above, thought it worth while to make ome additional experiments on the behaviour of the preparations in hypotonic, but alanced, solutions. Comparative experiments were also undertaken on preparaons from two other species: *Nereis diversicolor*, because of its power of osmotic egulation, and *Perinereis cultrifera*, a species closely related to the last, but much less ble to survive under brackish conditions. The results are described below, and iscussed as they arise, with reference to the relations between the different species nd their environments.

MATERIAL AND METHODS

The "isolated extrovert" of *Arenicola marina* has already been fully described Wells, 1937; Wells & Ledingham, 1940). It consists of the proboscis with a certain mount of oesophageal tissue attached, and gives a characteristic behaviour pattern, onsisting of alternating periods of vigorous rhythmic activity and rest.

We found it possible to make similar isolated extroverts—i.e. consisting corresponding parts—from *Nereis diversicolor* and *Perinereis cultrifera*. In no ca did we include the circum-oral nerve ring, the brain or any part of the ventral ner cord in an isolated extrovert. The preparations consisted of proboscis and oes phagus only, and contained the stomatogastric nervous system. Extroverts fro *Nereis* and *Perinereis* showed prolonged activity, but they were more delicate tha that of *Arenicola*—the preparation from *Nereis diversicolor* is particularly fine ar easily injured by careless handling. We therefore made other experiments, in th case of *Nereis* and *Perinereis*, on longitudinal strips of body wall cut from th anterior third of the worms. These strips were ventral and included the nerve cor (but no part of the nerve ring, as they began some segments behind the mouth They were active, robust and very satisfactory for experimental purposes.

Movements were recorded by means of light isotonic levers—lighter for th extroverts than for the body-wall strips, whose activity is somewhat improved by very moderate degree of stretching.

All the preparations used remain active for many hours when suspended in s water. We therefore assumed that sea water was a normal saline. Other fluids we made by diluting sea water with a thoroughly aerated solution of $NaHCO_3$ $M/400$ distilled water. This ensured constancy of pH at about the sea-water value. Con centrations are given throughout as percentage of sea water in the mixtures.

Most of the experiments, including all those incorporated in Figs. 4–6, were don in London at temperatures ranging from 14 to 20° C. A few, which were done durin a heat wave at temperatures over 20° C., gave unsatisfactory results and were di carded. The experiments on a sudden return from hypotonic to normal fluid we done partly in London and partly after our evacuation to Bangor, where the tempe atures were lower (down to 7° C.); this did not make any essential difference to th results described below.

GENERAL ACCOUNT OF THE EFFECTS OF DILUTION

The reactions of all the preparations used, to dilution of the bathing mediu are essentially alike, irrespective of species and of whether they are innervated b nerve cord or by stomatogastric system. This general similarity is interestin because, even within a single species, the tissues of extrovert and body wall sho striking differences in their reactions to drugs, such as adrenaline and acetylcholin (Wells, 1937; Wu, 1939).

If a preparation is bathed by sea water, which is then suddenly replaced by hypotonic mixture, its reaction consists of the following three phases (Fig.

(1) *Excitation*. The immediate response to the change is a brief period vigorous rhythmic action, with tonic contraction.

(2) *Inhibition*. After the excitation phase, the rhythm becomes inhibited. Th depth and duration of this inhibition vary with the new salinity, and also wit species. In low salinities, activity ceases altogether. In 50% sea water, *Perinere* tissues show complete stoppage for a time (Fig. 1), while those of the other tw

species show only irregularity and loss of vigour. Typically, the preparations of all species relax fully as they become inhibited, but with very low salinities they may remain in a state of partial contracture for many hours.

(3) *Accommodation.* If the new salinity is not below a "lower limit" characteristic of the species, spontaneous activity gradually reappears, sometimes after several hours' quiescence (Figs. 1, 2, 3).

Evidently, two distinct factors are affecting the tissue. The sudden change of salinity has evoked phases (1) and (2), which we term "shock effects". The new salinity may also have permanent effects which will be seen in phase (3) after the shock effects have passed off. Either a permanent effect or a shock effect might be of biological significance.

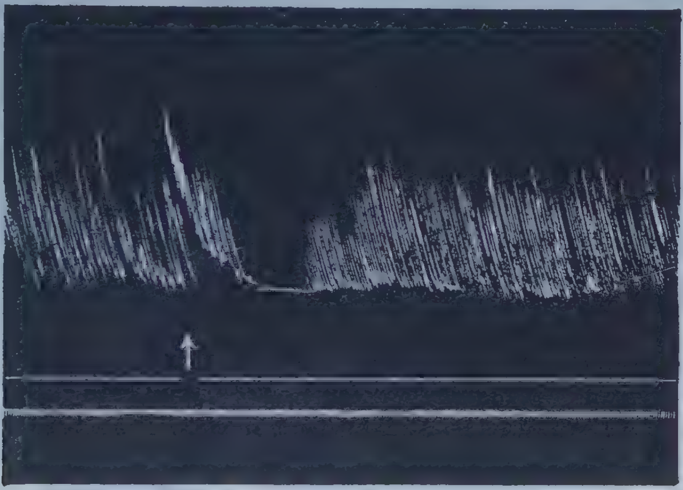


Fig. 1. *Perinereis* body wall. Effect of sudden change (at arrow) from sea water to 50 % sea water. *In all records*: read from left to right; upstroke of lever means contraction of preparation; time marked in minutes.

To meet this situation we worked according to the following plan. A number of experiments (*constant exposures*) were carried out as just described, i.e. the preparations were first allowed to settle down in sea water, then a hypotonic fluid was suddenly applied instead. The behaviour was then recorded for many hours in the new solution. In other experiments (*drifts*) the preparations were first mounted in sea water, and then buffered distilled water was slowly run in, in such a way that the salinity fell smoothly and steadily along an exponential curve. Fast and slow drifts were tried, the main object of these experiments being to find out the highest rate of dilution to which the preparations could be exposed without the appearance of shock effects. The details of the methods used are described in an Appendix (p. 350).

The chief results of the experiments are represented diagrammatically in Figs. 4–6. Each horizontal line summarizes the results of our constant exposures to that dilution. The initial brief excitation is omitted from the diagram, which therefore only shows phases (2) and (3) as listed above. It will be noted that the general for of the diagrams is the same for all of the three preparations shown, though the differ quantitatively, e.g. in the absolute magnitude of the lower limit. In all, th length of the inhibition phase increases greatly as that limit is approached.

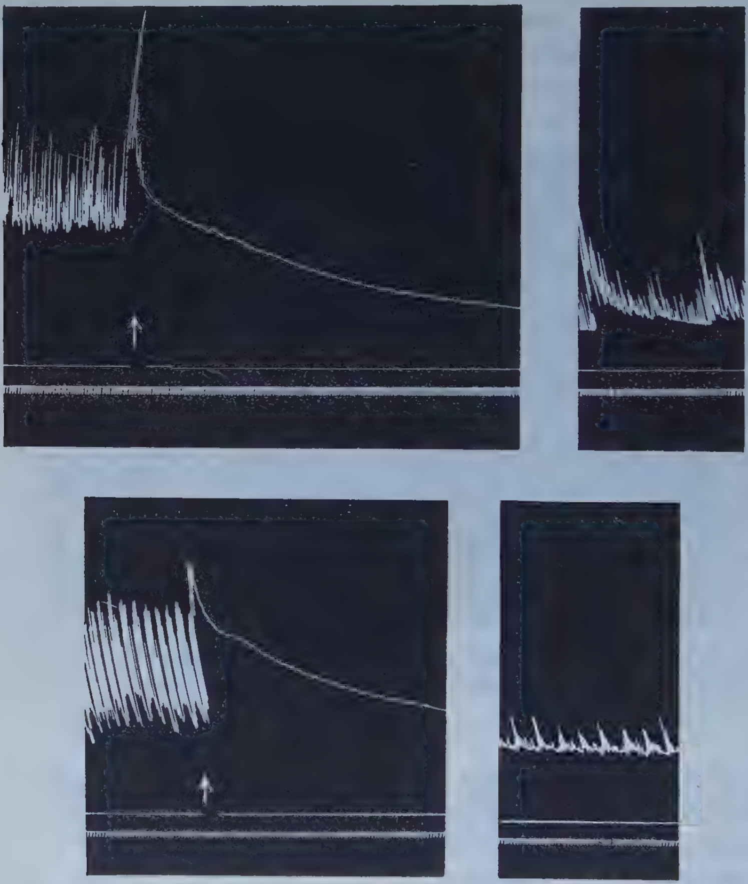
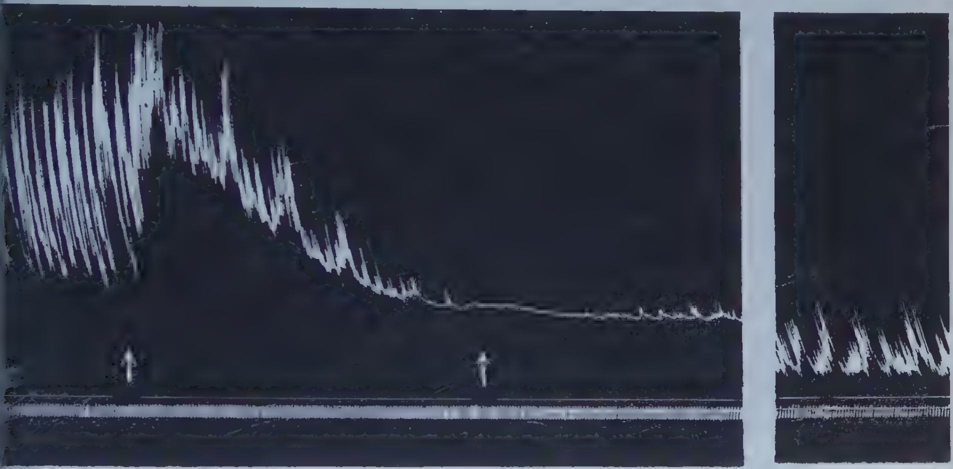


Fig. 2. Constant exposures to salinities near the lower limit. *Nereis* body wall above; *Arenicol* extrovert below. Both experiments begin in sea water, which is replaced at arrow by a hypotoni fluid—10 % for *Nereis*, 20 % for *Arenicola*. The second extract shows the behaviour, still in th hypotonic fluid, some time later. Note that accommodation has occurred. (Second extract is 1 hr long, and lasts from 12 to 13 hr. after the change of fluid in the *Nereis* experiment, and 21–22 hr. i *Arenicola*.)

The drift experiments appear on the diagrams as exponential curves. In th slowest drifts, the preparations are active down to a salinity roughly equal to th lower limit found in the constant exposure experiments. This indicates that th shock effects of sudden dilution are entirely temporary and reversible. If they included any permanent change in the tissue, the constant exposures and slow drift would give different lower limits. It may be objected that if yet slower drifts ha been done, the preparations might have remained active at even lower salinities. In e case of *Perinereis cultrifera* this is probably true. Unhappily, we could not test is possibility experimentally because, with the slowest drifts here recorded, the uration of the experiment is about as long as the isolated preparations will stand. he general form of the salinity diagrams strongly suggests that the lower limits ached by the two methods of experimentation are in fact identical.

In the more rapid drifts, as can be seen in the diagrams, inhibition appears above e true lower limit. It is easy to show, by stopping the drift when this inhibition ppears and recording the gradual reappearance of activity as the preparation ccommodates itself to the new salinity, that we are simply concerned here with a appearance of phase (2) of the reaction to sudden dilution. In very fast drifts hase (1) also appears, as a brief period of hyperactivity when the drift begins 'ig. 3).



g. 3. *Arenicola* extrovert; rapid drift experiment. Experiment starts in sea water. Drift starts at st arrow. At second arrow, 2 hr. later, the salinity has fallen to 28 % of sea water, and the drift is pped. Note that accommodation to the dilute medium then occurs. Second extract is 1 hr. long d begins 6 hr. after the drift stopped—still in the dilute medium.

The foregoing remarks describe the general behaviour common to all the prerations used. We now turn to certain special problems—first, the lower limits for e three different species, in relation to their ecological distribution, and then the ssible biological significance of the effects of too rapid change.

THE SALINITY TOLERANCE OF *ARENICOLA MARINA*

The experiments on *Arenicola* were mostly done in London on material from ymouth. A few points were completed at Bangor, using worms collected locally.

The lower limit for the extrovert of this species seems to lie near 15%. In nstant exposures to 20% rhythmic activity, of the characteristic intermittent type rmally shown by this preparation, invariably reappeared. In 10% no spontaneous activity was seen. Four extroverts were exposed to 15 % for 22 hr., with the follo ing results: one gave no activity at all; one showed very small, irregular contractio some hours after the drop, and after 16 hr. exposure began to give well-defined ou bursts of normal frequency (12 outbursts per hour in sea water, 10 in 15 %) but minute amplitude; two gave very slight, sluggish tone waves but no true rhythm.

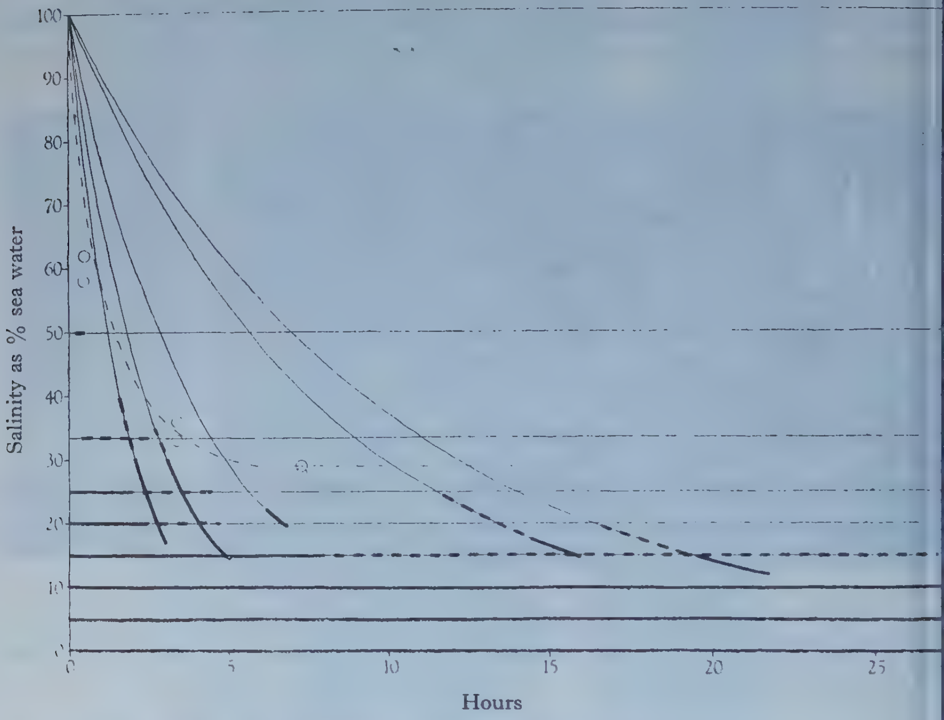


Fig. 4. Salinity diagram for the *Arenicola* extrovert. Horizontal lines represent constant exposu experiments, the drop from 100 to the lower percentage occurring at zero time. Exponentii represent the courses of the drift experiments. Thin continuous line means all preparations acti thick lines means all inhibited; thin with thick dashes means preparations vary. See text for t meaning of the circles, and thin broken exponential.

the slowest drifts, the existence of a lower limit in the range 15–20 % was confirm (Fig. 4). We noted, in the constant exposure experiments, that even after 22 hr. 10 %, the extrovert shows temporary excitement on returning to sea water and slight contracture can be elicited by K excess. After 22 hr. in 5 %, however, th seem to be dead.

At first sight the existence of a lower limit at 15–20 % seems to agree with t known facts about the distribution of the species. It occurs down to about 20–25 sea water; Krogh (1939, p. 47) quotes it as "living in nature at concentrations betwe ocean water and $8^{\circ}/_{\circ\circ}$". Its body fluids are isotonic with the environment at salinities (Schlieper, 1929). There is, however, a serious difficulty. At saliniti much higher than the lower limit our records show a considerable loss of amplitu vhose exact extent varies considerably from preparation to preparation. This occurs oth in constant exposures and in slow drifts. The amplitude in $33\frac{1}{3}$ % is usually bout a half, or even less, of its original value in sea water, while at 20–25 % it has allen to a quarter or a fifth (Fig. 2). It seems that the contractile mechanism, or erhaps some part of the conducting system, fails before the excitor mechanism. In low drifts, the contractions get smaller and smaller, but the characteristic inter- nittent behaviour pattern appears with normal timing, right down to the lowest alinity at which activity can be detected. Similarly, in constant exposures, when a reparation is emerging from the inhibition phase, the contractions first appear with ormal timing but with minute amplitude, which gradually increases until it stays t the level determined by the new salinity.

The result, then, of gradually diminishing the salinity to which an *Arenicola* xtrovert is exposed, is a steady falling away of amplitude, which finally leads to the omplete disappearance of activity. It is impossible to say at what point the prepara- ion becomes unfit for normal functional activity. The "useful limit" of the extro- ert is certainly well above the lower limit below which no spontaneous movements, owever small, can be seen. Our results seem therefore to conflict with the known istribution of the species, and we are faced by two alternatives. Either the reduced mplitude is due in some way to our experimental methods, and would not occur to a ignificant extent if a corresponding dilution of the body fluids of an intact worm ook place, or there is a physiological difference between the worms used by us and hose occurring in situations at the brackish end of the distribution range.

THE SALINITY TOLERANCE OF *NEREIS DIVERSICOLOR*

The *Nereis* body-wall strip gives vigorous records, in which it is generally ossible to detect periodic "activity outbursts", like those seen more clearly in *erinereis*, but more or less masked, in this case, by a background of practically ontinuous activity. The extrovert shows a rather irregular and variable rhythm, nd activity outbursts are usually evident in this case too.

The tissues of *Nereis diversicolor* are far more tolerant of low salinities than are hose of either of the other two species used in this work. The precise values seem, owever, to vary to some extent, either with season or with locality, for we got ather different results in the following two sets of experiments:

(1) Most of our work was done in London in June and July on Plymouth worms. oth body walls and extroverts were studied, and the results got with the former are ummarized in Fig. 5. The lower limit seems to lie near 10 %. In constant exposures, he following results were obtained with the body-wall strips: in 15 %, resumption f activity after inhibition lasting 4–5 hr.; in 10 %, four preparations became igorously active after 7–12 hr. inhibition, while four only became slightly active; in %, five preparations out of six showed no activity, while the sixth gave a few weak, low contractions at long intervals, beginning $15\frac{1}{2}$ hr. after the salinity drop. The esults got with the extrovert agreed with those of the body wall.

(2) We also made some experiments at Bangor in April, on worms from a ver brackish creek at Malltraeth, Anglesey. The animals were kept in sea water for som days before use, to make the conditions of the experiments as similar as possible t those of the others. Only body walls were used. The preparations were mor resistant than those just described. In 5 % sea water, all of three strips became quit vigorously active, although with rather reduced amplitude, after inhibition lastin from 10 to 13 hr. In 2½ % sea water no activity was seen. The duration of the shoc effects was also somewhat shorter, in animals of this group, than in those of Fig.

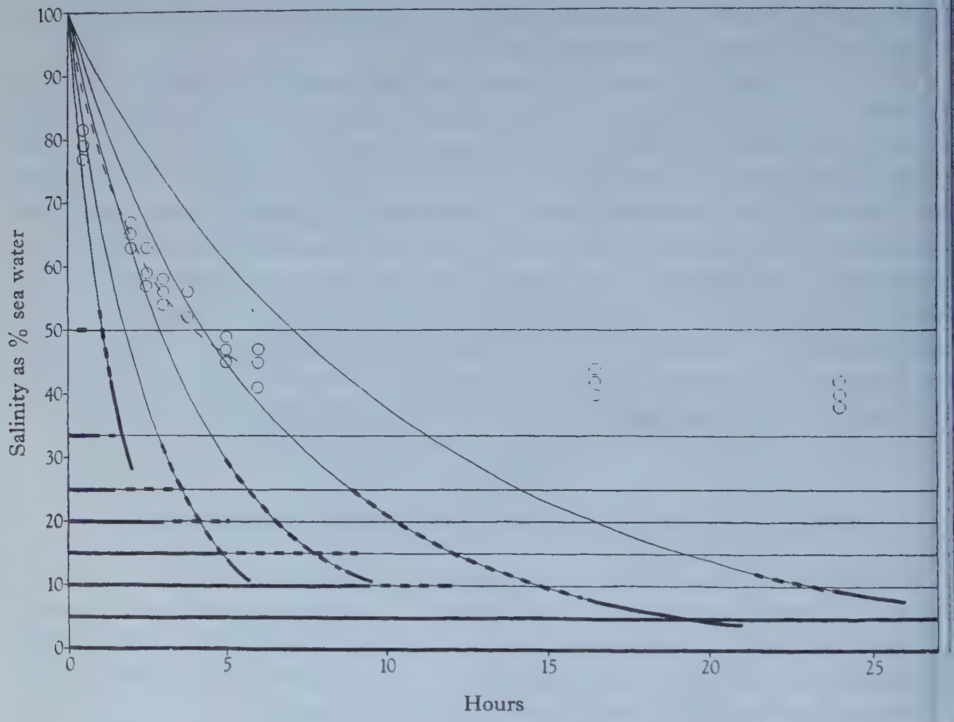


Fig. 5. Salinity diagram for the *Nereis* body-wall strip. Notation as Fig. 4.

The general conclusion, that the tissues of *N. diversicolor* are exceptional resistant to low salinities, is further supported by the fact that the preparations fro this species show no falling off in amplitude down to about 10%.

As is well known, *N. diversicolor* is found over a very wide salinity range. It i usually conspicuous in the fauna of brackish waters and penetrates far into the Balti (Heinen, 1911); it occurs at all intertidal levels (Thamdrup, 1935); it can also live i highly concentrated saline waters (Hesse *et al.* 1937; Fauvel, 1923). According t Schlieper (1929) it can be kept alive for 14 days in fresh water. This euryhalinity ha been developed in two ways: firstly, the worm is able to maintain a considerabl degree of hypertonicity even in very dilute media (Schlieper, 1929; Beadle, 193

Zenkewitch, 1938), and secondly, its tissues have acquired an exceptional degree of resistance to hypotonic bathing fluids. Indeed, at first sight the ability of its neuromuscular mechanisms to function down to 5–10 % of sea water would seem to be unnecessary, since even in water of $\Delta = -0{\cdot}04^\circ$ it can maintain an internal osmotic pressure of $\Delta = -0{\cdot}5^\circ$ (Schlieper, 1929), and we seem to be confronted with another instance of the phenomenon of "duplication of mechanism" to which Barcroft (1934) has drawn attention. Possibly the high degree of resistance of the tissues is useful if the external surfaces of the animal are injured, or if it is exposed to high temperatures—a factor which has been shown greatly to reduce the effectiveness of osmoregulation in various crustacean species (Widman, 1935; Otto, 1937) and of volume regulation in *N. diversicolor* (Beadle, private communication).

THE SALINITY TOLERANCE OF *PERINEREIS CULTRIFERA*

The *Perinereis* body wall typically gave a rhythmic pattern in which more or less well-defined activity outbursts were conspicuous—a fact whose possible biological significance has been discussed elsewhere (Wells, 1939). The extrovert gave records like those of the *Nereis* extrovert, except that the rhythmic outbursts were usually more pronounced.

The tissues of *Perinereis* are even more sensitive to dilution than those of *Arenicola*, but in this case, as in *Nereis*, we have found slight differences between two batches of animals. Both were sent from Cullercoats. The first was studied in London in July, the second in Bangor in April.

Fig. 6 was drawn from the results of the first batch. Both body wall and extrovert showed a lower limit between 20 and 25 %. A curious feature of this batch was the extraordinarily long inhibition period shown after downward change. In the case of the body-wall strips, even 50 % produced complete quiescence for over an hour, while of two strips exposed to 25 %, one was inhibited for 14½ hr. and the other for 20½, before accommodation resulted in the resumption of activity. The drift seems to have been too fast for the tissues of this batch; it was done on two body-wall strips and two extroverts; the former became inactive at 30 and 23 %, and the latter at 33 and 25 %. Probably this is another expression of their great sensitivity to shock, and a slower drift would have brought them all to 20–25 % before activity ceased.

In the second batch only body walls were used. They became active in 20 but not in 15 %. This group showed more resistance to dilution and less marked shock effects than the other. Both, however, were more sensitive than the extrovert of *Arenicola marina*.

Perinereis cultrifera has a very limited power of penetrating into water of low salinity. It generally lives fairly far down the beach. In the north-eastern Atlantic, however, it penetrates to some extent into estuaries (Hesse *et al.* 1937), and it occurs at Sevastopol at a salinity of $18\,^\circ/_{\circ\circ}$ (Zenkewitch, 1938). It is found in the Kattegat but not in the Baltic (Heinen, 1911).

The body fluid of our worms was approximately isotonic with the external medium. This statement is based on measurements kindly made for us by Dr N. F Panikkar, using A. V. Hill's thermocouple technique. Worms from our Londo batch were left overnight in 25 % sea water. The following values were then foun expressed as percentage of NaCl in a solution isotonic with the fluid investigate external medium, 0·834; body fluids of three worms, 0·848, 0·857, 0·874 respectivel There was therefore a very slight degree of hypertonicity.

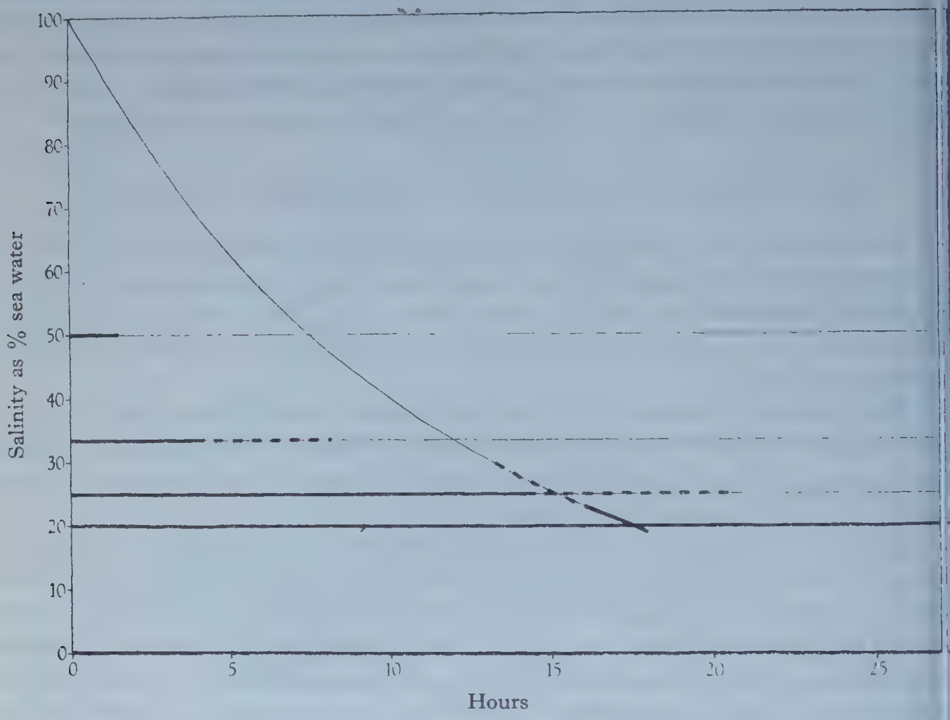


Fig. 6. Salinity diagram for the *Perinereis* body-wall strip. Notation as Fig. 4.

To judge by our records, the activity of the preparations, even of the mo sensitive batch, is not seriously interfered with down to about 25 %. Even assumin complete isotonicity of internal and external media, this would allow the worms t live in fairly dilute waters, including Sevastopol and Kattegat water. The story i however, more complicated than these data suggest. According to Zenkewitc (1938), Sevastopol worms can maintain a markedly hypertonic internal environme over a limited range of external salinities; in 25 % sea water his animals showed th ability to a much greater extent than did ours, as measured by Panikkar. The seems, therefore, to be a physiological difference between the two groups o animals. Although the Cullercoats' worms could probably live in Sevastopol wate if they were transferred to it slowly enough, the Sevastopol worms seem to hav developed special adaptations to their environment.

THE BIOLOGICAL SIGNIFICANCE OF SHOCK EFFECTS

Under estuarine conditions, the salinity of the environment fluctuates, and we must now enquire whether the worms are likely to encounter salinity changes in heir natural habitats, rapid and great enough to produce shock effects in their issues, like those seen in *in vitro* experiments.

Any salinity change in the environment will of course be "damped" before it cts on the tissues, since the internal osmotic pressure will only follow the external fter a certain time-lag. Fortunately, L. C. Beadle (1937) has published measurements of the rate at which the internal osmotic pressure of *Nereis diversicolor* falls when the whole worm is suddenly transferred from 100 to 25 % sea water at 15° C. His measurements are included as circles in Fig. 5. Each point represents the vapour pressure of the body fluid of a single worm, using A. V. Hill's thermocouple method. Mr Beadle has also kindly allowed us to incorporate, in Fig. 4, unpublished measurements made by him in the same way with *Arenicola marina*. In this case also, the worms were transferred from 100 to 25 % sea water but the temperature was 21° C. t will be seen that the curve falls more steeply in the latter species, which lacks an osmoregulator mechanism.

To find out what these salinity-time curves mean to the tissues of the worms, we "aimed" drift experiments through Beadle's points. This was made difficult by the act that the points do not lie on an exponential curve; they are too steep at the beginning. In the case of *Nereis diversicolor*, we decided that a close enough pproximation could be got by running 40 % sea water into 100 % at an appropriate ate. The resulting exponential lies fairly near the points (thin broken line in Fig. 5). n the case of *Arenicola marina*, we started with the preparations in 100 % sea water nd ran in 28 %, using two Mariotte bottles and capillaries which delivered at pproximately equal rates. One was used during the whole drift and the other for he first half hour only, to steepen the first part of the curve. Even with this method our curve was less steep at the beginning than Beadle's; but the agreement is fairly good (thin broken line in Fig. 4). The drift was stopped after 6 hr., at which time the salinity had fallen to 29 %, and the record was continued with the preparations still at that strength.

When a whole *A. marina* is placed from pure into dilute sea water it becomes very estless, and makes frequent gulping and burrowing movements. After a little while it becomes more passive. This may help to account for the rapid water uptake at the beginning of the immersion period, shown by the great steepness of the first part of Beadle's curve.

If *Arenicola* extroverts are drifted along the *Arenicola* dilution curve (i.e. the ine broken line of Fig. 4), the disturbance of function is at no stage very severe. There is initial excitement, resulting in continuous activity for some minutes, and from about 55 % the normal pattern is somewhat disturbed; but there is no paralysis at any stage. On stopping the drift at 29 %, the normal behaviour pattern was very rapidly resumed. Clearly, when whole worms are suddenly transferred from 100 to 25 % sea water, their internal osmotic pressure drops rapidly enough to disturb the normal functional pattern to some extent, but not rapidly enough to cause inhibitio It should be noted that in this discussion we are limiting ourselves to the actu effects of osmotic pressure, and leaving aside any consideration of turgor or oth mechanical effects of the volume of fluid taken up; in the case of *A. marina*, the effects have been described by Reid (1929*a*).

If *Nereis* body-wall strips are subjected to the same drift, i.e. the fine broken li of Fig. 4, they show rather more effect. The amplitude drops to about a quarter its original value, and then recovers when the drift is stopped. The picture is ve different if the *Nereis* dilution curve is followed. Two body-wall strips, and tw extroverts, were drifted along the fine broken line of Fig. 5. They all remain vigorously active throughout the experiment, and at no stage was there the slighte sign of disturbance. This shows that when whole worms are suddenly transferr from 100 to 25% sea water, their internal osmotic pressure falls too slowly to ha any effect on their neuromuscular mechanisms. The result of drifting *Ner* preparations along the *Arenicola* curve shows that *Nereis* has its osmoregulat mechanism to thank for its immunity against osmotic shock effects.

The osmotic change in sudden transfer from 100 to 25% sea water is probab more violent than any that the worms encounter under natural conditions. Measur ments of salinity variations in the interstitial water of the mud in which *Nereis* an *Arenicola* burrow have been published by Bruce (1928), Reid (1929*b*, 1932) an Thamdrup (1935). At the depth to which the worms burrow the changes are n great. It should, however, be remembered that the water inside the burrow is n identical in composition with the interstitial water of the mud. Both *Nereis* an *Arenicola* ventilate their burrows vigorously (van Dam, 1937; Lindroth, 1938), an the existence of a yellow layer of oxidized sand in the wall of the burrow shows th the water inside differs, at least as regards oxygen content, from that outside. We d not know what happens inside a worm burrow when heavy rain falls at low tid However, the results of our experiments based on Beadle's measurements make almost incredible that the salinity changes are severe enough to produce osmot shock in the tissues of these two species.

The tissues of *Perinereis* are more sensitive to sudden dilution than those *Arenicola* or *Nereis*; unhappily, data on the "damping" effect are not available f *Perinereis*, so we are unable to extend our analysis to this case.

THE EFFECT OF RETURN FROM HYPOTONIC SOLUTIONS TO SEA WATER

Hitherto, we have been concerned exclusively with the effects of downwa salinity change. A number of experiments were also made with upward change using the *Arenicola* extrovert and the *Nereis diversicolor* body wall. The results we peculiar, for in the case of downward changes all the different preparations ga essentially the same type of response; with upward changes on the other hand, t responses seem to differ in kind. With the *Nereis* body wall, we find that a sudd return to sea water evokes a cycle of reactions very like those seen after sudd downward change (Fig. 7). As one would expect, these shock effects can be avoided by slowly drifting upwards from the hypotonic to the normal fluid. The *Arenicola* extrovert, on the other hand, shows temporary excitement, whose duration is short as regards the rhythmic mechanism and somewhat longer as regards tone (Fig. 7); it does not show the shock inhibition, which is such a conspicuous feature of the *Nereis* records. This difference was consistently found in Plymouth and Bangor specimens of both species.

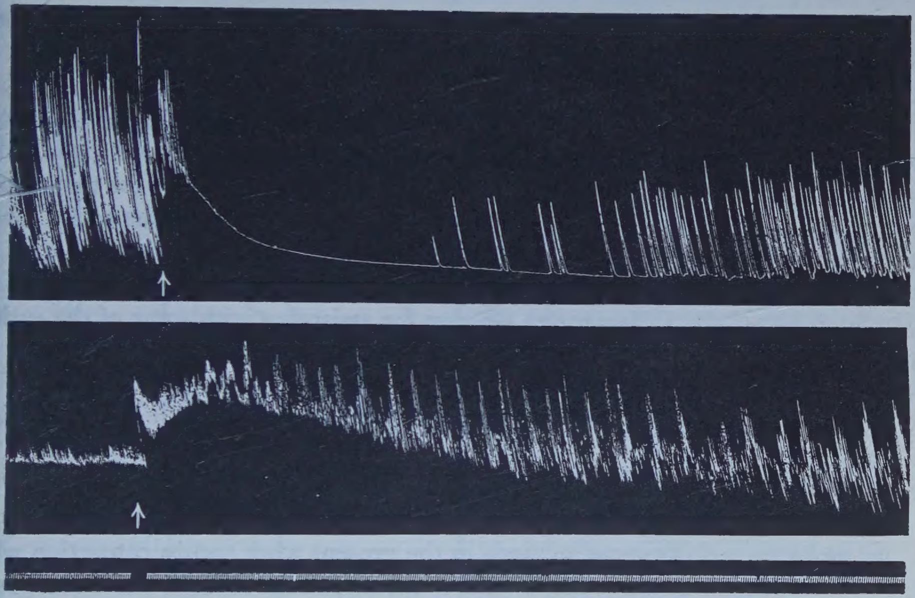


Fig. 7. Return from hypotonic to normal sea water. *Nereis* body wall above; *Arenicola* extrovert below. In both cases the record starts in 25 % (preparations thoroughly accommodated), and this is replaced by 100 % at arrow.

SUMMARY

1. The reactions of isolated rhythmic preparations from *Arenicola marina*, *Nereis diversicolor* and *Perinereis cultrifera* to hypotonic salines are described.

2. The preparations used were (1) the "isolated extrovert" of all three species, (2) ventral longitudinal body-wall strips of the two last named. All these preparations are essentially alike in their reactions to dilution of the bathing medium.

3. On abruptly changing from sea water to a hypotonic fluid, responses of the following general type are seen: first, brief excitement; then a phase of more or less complete inhibition; finally, provided the hypotonic fluid is not below a lower salinity limit characteristic of the preparation, gradual return of activity as the preparation accommodates itself to the new medium. The first two phases are shock effects of sudden dilution. The inhibition phase may last for many hours.

4. Preparations were exposed to salinities which fell gradually at various speeds.

From the results of these experiments it is inferred that shock effects of rapid chang are unlikely to be evoked under natural conditions, at least in *Arenicola* and *Nerei*

5. The lower salinity limits for spontaneous activity in the tissues of the variou species are: *Perinereis cultrifera*, 20–25 % sea water; *Arenicola marina*, 15–20 % *Nereis diversicolor*, 5–10 %. These results are discussed with reference to th ability to live in brackish water.

6. On suddenly returning from a hypotonic fluid to normal the responses var There may be relatively slight excitation (*Arenicola marina* extrovert) or a cycle o excitation—inhibition—accommodation like that evoked by a sudden downwar change (*Nereis diversicolor* body wall).

The work was begun at University College, London, and completed after ou evacuation to the University College of North Wales, Bangor. We are glad of thi opportunity of expressing our thanks to Prof. Brambell and his staff for the hospit able welcome with which we were received. Our thanks are also due to Dr L. C Beadle for kindly allowing us to use his unpublished measurements in Fig. 2, t Dr N. K. Panikkar for determining the vapour pressure of *Perinereis cultrifer* body fluid, and to Miss Clementina Gordon for assistance with some of the earlie experiments.

APPENDIX

Simple methods for exposing isolated organs to sudden and gradual changes in the chemical environment

As we have used the same methods in the present work and in connexion wit other problems, we publish the details as an Appendix to facilitate subsequen reference. Our experiments were of two types, which we term "constant exposures and "drifts".

Constant exposures

In this case the changes of solution are made as suddenly as possible, bu between changes the preparations are exposed to constant environments.

The device shown on the left of Fig. 8 has proved very convenient for thi purpose. The preparation (dotted) is suspended inside tube *A*, which has an ope cup-shaped top and a sloping side-arm *B*, along which surplus fluid can run away Aeration is done through capillary tube *C*, blown to the lower end of *A*. Inflow tub *D* is constricted where it fits into *A*, and the dimensions of the two tubes are suc that a centimetre of pressure tubing, run on to *D*, acts at this point as a rubbe stopper. Above, *D* ends in a hook to which the preparation is attached. Just belo this hook, a hole in the side of *D* allows new fluid to enter. By running in a generou amount of the new fluid, very rapid and complete changes can be made withou interrupting the record. Thread *L* runs to the recording lever.

For constant exposure experiments of long duration, a vessel of larger capacity i desirable, so that the bathing fluid need not be frequently renewed. In this case i has been our practice to mount preparations in the cylindrical jars used for drift experiments (see below), and to change the fluid simply by siphoning off the old as completely as possible and then pouring in the new through a tube running to the bottom of the jar. An excess of the new fluid was always run in, so that some of it escaped over the rim of the jar and washed away any residue of the old, and the completeness of the change was always checked by titration of some appropriate constituent of the fluids. This method has the drawback that the record is somewhat defaced at the moment of change.

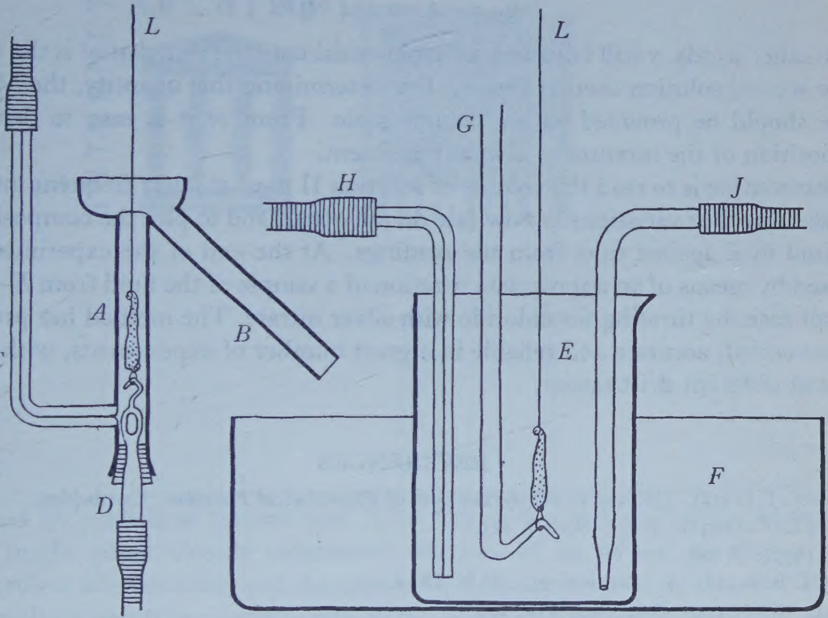


Fig. 8. See text.

Drifts

In experiments of this type the preparation is exposed to a fluid whose composition "drifts" slowly and steadily along an accurately predictable curve, whose steepness can be varied at will.

Cylindrical jar *E* (Fig. 8) stands in an outer vessel *F*, and contains the preparation attached to glass hook *G*. Thread *L* runs to the recording lever. (We generally used jars of capacity 385 c.c., in which two or three preparations could be mounted and recorded simultaneously.) At the beginning of the experiment, *E* is filled to overflowing with solution I (generally sea water in our case, which is a suitable "normal" fluid for polychaete preparations). When the preparation has settled down, the "drift" begins. A second solution is run in from a Mariotte bottle through a fine capillary *H*, i.e. at a constant, slow rate. The Mariotte bottle should be connected to *H* with stout pressure tubing and the flow rate should be regulated by adjusting its height (a screw clamp on the tubing leads to irregularities). Th contents of E are kept continually mixed by air entering through J, and, as fast a the second solution flows in, a mixture of the two fluids escapes over the rim of into F.

In this system, if V = volume of E, v = flow rate, t = time, x = amount of solution left in E, as percentage of its volume, then

$$dx/dt = -vx/V,$$

or, since $x = 100$ when $t = 0$,

$$\log_{10} x = 2 - 0{\cdot}434\, vt/V.$$

In other words, x will fall along an exponential curve. Note that vt is the volum of the second solution used at time t. For determining this quantity, the Mariott bottle should be provided with a volume scale. From vt it is easy to derive th composition of the mixture in E at any moment.

Our routine is to read the volume of solution II used at fairly frequent interval to make sure that variations in flow rate do not occur, and to plot the composition the fluid in E against time from the readings. At the end of the experiment, x checked by means of an appropriate titration of a sample of the fluid from E—in th present case, by titrating for chloride with silver nitrate. The method has proved be convenient, accurate and reliable in a great number of experiments, with a wid range of different drift rates.

REFERENCES

BARCROFT, J. (1934). *Features in the Architecture of Physiological Function.* Cambridge.
BEADLE, L. C. (1931). *J. exp. Biol.* **8**, 211.
—— (1937). *J. exp. Biol.* **14**, 56.
BRUCE, J. R. (1928). *J. Mar. biol. Ass. U.K.* **15**, 5.
DAM, L. VAN (1937). *Zool. Anz.* **118**, 122.
FAUVEL, P. (1923). Polychètes errantes. In *Faune de France.* Paris.
HEINEN, A. (1911). *Wiss. Meeresuntersuch.* (Abt. Kiel), N.F. **13**, 1.
HESSE, R., ALLEE, W. C. & SCHMIDT, K. P. (1937). *Ecological Animal Geography.* New York an London.
KROGH, A. (1939). *Osmotic Regulation in Aquatic Animals.* Cambridge.
LINDROTH, A. (1938). *Zool. Bidr. Uppsala,* **17**, 367.
OTTO, I. P. (1937). *Zool. Anz.* **119**, 98.
REID, D. M. (1929*a*). *J. Mar. biol. Ass. U.K.* **16**, 109.
—— (1929*b*). *J. Mar. biol. Ass. U.K.* **16**, 609.
—— (1932). *J. Mar. biol. Ass. U.K.* **18**, 299.
SAYLES, L. P. (1935). *Biol. Bull. Wood's Hole,* **69**, 233.
SCHLIEPER, C. (1929). *Z. vergl. Physiol.* **9**, 478.
THAMDRUP, H. M. (1935). *Medd. Komm. Havundersøg., Kbh.,* **10**, 2.
WELLS, G. P. (1937). *J. exp. Biol.* **14**, 117.
—— (1939). *Nature, Lond.,* **144**, 940.
WELLS, G. P. & LEDINGHAM, I. C. (1940). *J. exp. Biol.* (in the press).
WIDMAN, E. (1935). *Z. wiss. Zool.* **147**.
WU, K. S. (1939). *J. exp. Biol.* **16**, 251.
ZENKEWITCH, L. (1938). *Zool. Zh.* **17**, 845, 976.